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THE OVULE AND FEMALE GAMETOPHYTE OF DIOON.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXXVI.

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(WITH NINE FIGURES AND PLATES XIII–XV)

ONLY two of the nine genera of cycads have received any considerable attention from morphologists. In these two, Cycas and Zamia, the life history is fairly well known and many of its phases have been studied in great detail. In Stangeria the development of the sporangia has been investigated, and work on the remaining six genera is fragmentary.

Through the courtesy of the Botanical Society of America[1] the writer was enabled to visit the Mexican tropics for the purpose of securing material of Dioon and Ceratozamia. The hearty cooperation of Governor TEODORO A. DEHESA, who is an active educator as well as a statesman, made it possible to collect an abundance of material in a very short time. Mr. ALEXANDER M. GAW, of the State Bureau of Information, Xalapa, Mexico, had many collections of material brought into Xalapa from the field and forwarded to me after my return to Chicago. To both of these gentlemen I wish to express my sincere thanks, since the investigation would have been very limited without their efficient assistance. The photographs used were made by Dr. W. J. G. LAND.

The subject will be treated under the following heads: I. Dioon in the field; II. Material and methods; III. The ovule; IV. The female gametophyte; V. The microsporangium; VI.

[1] The grant was made in December 1904 by the Botanical Society, at the St. Louis meeting of the A. A. A. S., and was for the purpose of securing material for a morphological study of Dioon and Ceratozamia.

Male gametophyte; VII. Fertilization; VIII. The embryo and seedling. The first four are presented in the present paper; the remaining subjects have been investigated and the results will be published soon.

I. DIOON IN THE FIELD.

Dioon edule occurs in great abundance at Chavarrillo, about 25^{km} east of Xalapa, the capital of the State of Vera Cruz. During March 1904 I made frequent trips through this region and, besides the tedious work of preparing material, was able to make a few observations upon the plant as it occurs in the field. A second trip, devoted largely to field study of both Dioon and Ceratozamia, was made in September 1906. As the results of this trip will be published in a separate paper, only a brief description of the general appearance of Dioon is given at this time.

Associated with Dioon are occasional specimens of a large Opuntia. Orchids and Tillandsias are not infrequent on the trees at the bottom of ravines, though neither of these plants flourish on the rocky slope where Dioon is at its best. In the lower portions of ravines several species of Selaginella are abundant. Dioon is so abundant on the rocky slopes that from a favorable point of view as many as a hundred plants may be counted. Seedlings are numerous and one always meets these and then small crowns before coming upon plants large enough to bear cones. In spite of the seedlings, it is doubtful whether the limited range is being extended.

In habit Dioon resembles *Cycas revoluta* (*fig. 1*). Some call it the "Dolores palm;" but the natives are more likely to call it *Tio Tamal* ("Uncle Tamal"), because they use the large endosperm in making *tamales*. The largest plant measured had a trunk nearly 3^{m} in height, and plants 1–1.5^{m} in height are not infrequent. The trunk often rises obliquely, as shown in the figure, and as noted in taxonomic descriptions.

The age of individuals.—Even in the largest plants the leaf scars are perfectly distinct over the entire surface, so that it is possible to determine with almost absolute accuracy the total number of leaves which a plant has borne. Professor LUIS MURILLO has estimated the age of individual plants by noting the number of leaves in a crown, the duration of the crowns (two years), and the entire number of

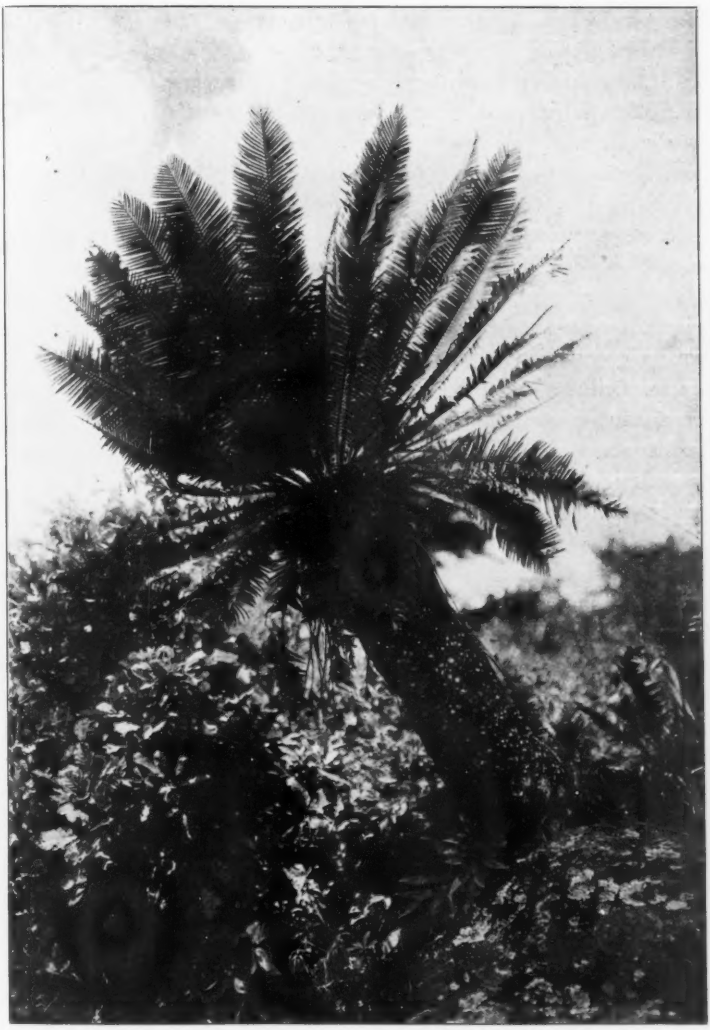

FIG. 1.—Ovulate plant of *Dioon edule* on rocky hillside at Chavarrillo, Mexico; the trunk is 1.5^{m} high.

leaf scars. From such data, the age of one plant with a trunk 1.45^{m} in height and 26^{cm} in diameter was estimated at 970 years. A small plant only 21^{cm} in height is known to have been in cultivation more than 40 years, and was presumably a fine specimen when brought in from the field. In cultivation a crown may persist more than two years, for the crown of a plant in the Washington Park (Chicago) conservatory has remained vigorous for at least five years. It seems probable that MURILLO's estimate is conservative, and that many of the large plants have reached an age of more than a thousand years.

The trunk.—The trunk is always straight and shows no external evidence of branching; among the thousands of plants observed, one only showing definite branching. The specimen had a Y-shaped trunk, each arm of the Y bearing a large crown. A few specimens were seen with two, three, or four crowns; and one plant had five and another six. The extra crowns do not come from loose buds which might become detached, as is so commonly the case in *Cycas revoluta*, but are all interlocked at the top of the stem. Some of the extra crowns are probably due to injuries received in the removal of cones, while others originate from the germination of seeds which had not fallen to the ground but had remained in the nest of the crown. Buds like those of *Cycas revoluta* also occur; occasionally they are found near the bases of old trunks, and at the top they are quite common. Some of these buds have well-developed crowns and would doubtless grow into independent plants if they should become detached and gain a suitable foothold. Root tubercles were observed but they were infrequent.

The ovulate cones.—In 1904 fruiting plants were not abundant; among plants large enough to bear cones not more than one in ten was in fruit, and among these staminate plants were more numerous than ovulate. In 1906 at least one-third of the larger plants bore cones. I was informed that a plant fruits every other year, and judging from the proportion of plants bearing cones in 1906 this would seem true; but an estimate based upon the proportion of plants bearing cones in 1904 would make the interval very much longer. The ovulate cones are large and ovoid (*figs. 2, 3*). Cones weighing 5^{kg} are common; and one large cone weighed 6^{kg} after

FIG. 2. —Ovulate cone 30cm in length; some leaves have been trimmed away.

it arrived in Chicago, more than two weeks after it had been taken from the plant. This was a March cone and probably would have gained another kilo before June. The length of the mature

cone is 20–30^{cm}, and the greatest diameter is 12–20^{cm}. Young cones picked November 1, 1904, five months before the cones reach full size, weighed 1.5–2^{kg} after reaching Chicago. The single cone rests snugly on the plant at the center of the crown of leaves, the peduncle being entirely concealed, so that it is difficult to remove

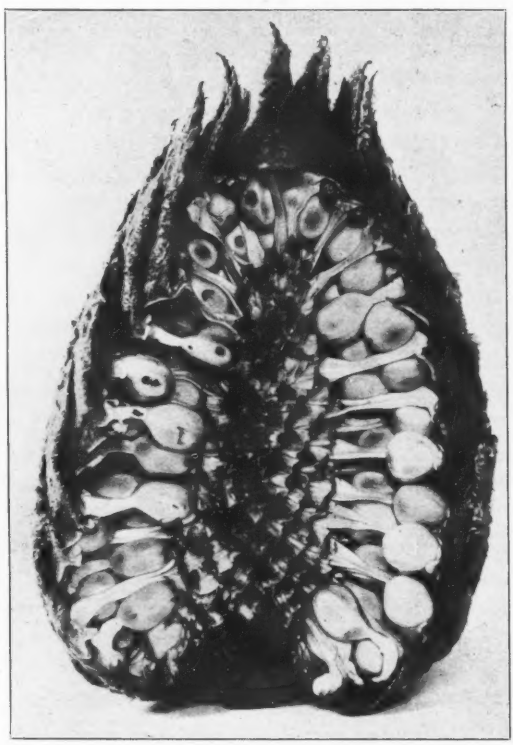

FIG. 3.—Ovulate cone 33^{cm} in length, showing position of ovules.

the cone with a knife. The easiest way is to grasp the cone with both hands and push it firmly to one side until the peduncle snaps with a clean transverse break. After the seeds are ripe the peduncle elongates somewhat, so that the cone often leans to one side, perhaps facilitating the dispersal of seeds. There is a well-developed abscis-

sion layer at the base of the petiole of the sporophyll, so that the whole sporophyll drops easily when the seeds are ripe. A second abscission layer, which separates the ovules from the sporophyll, does not mature until a much later period.

II. MATERIAL AND METHODS.

In March the ovulate cone has almost reached its full size, and the staminate cone of the same season has decayed. The first staminate cones were sent from the field on June 30, 1904. These showed the four microspores still held together by the wall of the spore mother cell. From this point to the discharge of the motile sperms a complete series in the development of the male gametophyte was secured. In the female gametophyte the series is complete from the appearance of the archegonium initial to the germination of the seed. The series showing the development of the embryo is very complete.

Nearly all the material was fixed in chrom-acetic acid in various proportions, with or without the addition of osmic acid. The following formula is excellent for the pollen tube structures and for young ovules: chromic acid, 1^{gm}; glacial acetic acid, 4^{cc}; 1 per cent osmic acid, 2^{cc}; water, 100^{cc}. This fluid will not penetrate the microsporangia, and with older ovules it not only causes some plasmolysis but makes the endosperm very hard to cut. After the endosperm has become starchy, better results were secured by using a slight modification of a formula suggested by Dr. LYNDS JONES: 50 per cent. alcohol, 100^{cc}; commercial formalin, 6^{cc}. This reagent penetrates well and fixes rapidly. Iron-alum haematoxylin stains brilliantly after it, but the safranin gentian-violet orange combination does not give as bright a stain as with material fixed in chromic solutions. After this reagent, the starchy endosperm is not so hard to cut. After the stony coat of the ovule has become hard, it is almost impossible to cut it with any knife without injuring the archegonia. For such stages the ovules were sawed in two transversely with a fine wire-like fret saw. The upper part of the endosperm with its archegonia was then trimmed into suitable shape for cutting. The extremely thin blade of the Gillette safety razor, soldered to any suitable handle, is particularly adapted for such trimming, since it

causes no damaging pressure as does the wedge-shaped blade of a scalpel or ordinary razor. The nucellus usually remains within the cup-like top of the ovule. Four strokes with a sharp scalpel will cut through the nucellus against the stony background and remove a piece containing the pollen tubes. Methods for studying the living sperms will be given in a second paper. For showing the grosser structures of the ovules, sections 4 or 5^{mm} in thickness were dehydrated and then cleared in xylol. For tracing the vascular bundles, the pseudo-stalks of ovules were cut under water and the bases were placed in eosin. The outer bundles soon become conspicuous on the surface, and the inner bundles are easily traced by removing the endosperm and scraping away the greater part of the inner fleshy layer of the integument.

Most of the sections were stained in safranin and gentian-violet; some were stained in iron-alum haemotoxylin. Magdala red and anilin blue proved to be a good combination, especially for pollen-tube structures, and it is quite convenient, since no clearing agent is necessary, the slides being taken directly from the absolute alcohol and mounted in Venetian turpentine. Overstaining in the Magdala red can be corrected, even after the cover glass is in place, by exposing the slide to direct sunlight for a short time.

III. MEGASPOROPHYLLS AND OVULES.

No other cycad, except Cycas, has such large and leaf-like megasporophylls as Dioon (*figs. 4–6*). Both the leaf-like character of the megasporophylls and their comparatively loose association in the cone are more suggestive of Cycas than of Zamia or Ceratozamia, which are the geographical neighbors of Dioon. The remaining occidental cycad, Microcycas, is too imperfectly described to allow any comparison of the cones.

In Dioon the sporophylls at the base of the ovulate cone are yellowish or greenish, with little hair except along the central portion of the back. There are four or five turns of the spiral of these sporophylls, followed by one or two turns of somewhat hairy sporophylls. The remaining sporophylls are densely covered with long brown hairs, the lower ones on the back and edges and a small portion of the upper part of the inner face, and the rest not only upon the back

and edges but upon the entire upper half of the inner face. This gives the whole cone a brown color and a densely hairy appearance. It would seem impossible for the cone to be wetted by rains. The changes in temperature in this region are so slight that the hairy condition could hardly be related to this factor.

The lower greenish sporophylls never bear ovules or even primordia of them. The next sporophylls have occasional primordia, but it is only when the larger sporophylls are reached that normal ovules

FIG. 4.—Ovulate sporophyll, back and side view. ×½.

FIG. 5.—Ovulate sporophyll, inside view; ovules sessile. ×½.

FIG. 6.—Ovulate sporophyll, abaxial side; one ovule with pseudo-stalk, the other sessile. ×½.

appear. Even the uppermost sporophylls bear ovules which sometimes ripen into seeds. The uppermost sporophyll is almost circular in transverse section. Its two ovules usually abort, as do those of the next sporophyll below it, but from this point, down to the sterile sporophylls at the base of the cone, each sporophyll bears two ovules which may develop into seeds. The ovules in a cone number about 100–300, and probably 200 ripe seeds is a liberal estimate for the larger cones. Where plants are isolated and pollination is uncertain, there are few ripe seeds, or even none at all. It would not be safe to say that the ovules of Dioon do not attain their full size unless pollination has taken place, for it is well known that the ovules of

other cycads reach their full size without the stimulus of pollination. However, the only greenhouse cone of Dioon which has come to my notice had only abortive ovules, and in the field the ovulate cones at any considerable distance from staminate plants are likely to contain only abortive ovules. Such ovules have not been pollinated. In cones bearing ripe seeds the abortive ovules have usually been pollinated, but have failed to develop from lack of room or of nutrition. It is easy to determine whether pollination has taken place, since the course of the pollen tubes is marked by conspicuous brown lines upon the nucellus.

The youngest ovules secured in 1904 were sent from the field November 1. These were 1^{cm} in length and showed the archegonium initials. Only one small cone had ovules as young as this, the ovules in other cones of this date measuring 1.5^{cm} in length and showing the central cell of the archegonium. November 14, six weeks after pollination, the average length of the ovules is about 2.3^{cm}; in the following spring (April 3), when the ovules had reached their full size, the largest ones measured 4^{cm} in length and 2.2^{cm} in diameter. The average length is about 3^{cm}, and the diameter 2^{cm}. Many ovules are nearly spherical, measuring about 3^{cm} in length and 2.8^{cm} in diameter. The ovules are perfectly smooth, and until nearly ripe are white, but become cream-colored or yellowish when exposed to the air; at maturity they have an orange color which contrasts sharply with the pale yellow of the naked portion of the sporophyll. They are sessile, but many of them appear to be stalked because strains due to the growth of the sporophylls and ovules draw out the base of the sporophyll into a stalk-like structure (*fig. 5*).

The ovule of Dioon reaches its full size before the stony layer becomes hard enough to occasion any serious difficulty in sectioning. Median longitudinal sections 3 or 4^{mm} in thickness, well dehydrated and cleared, but not stained, are best for a study of the general topography (*fig. 10*); while fresh ovules whose bundles have taken up eosin are most convenient for tracing the vascular system (*figs. 7, 8, 9*). The epidermis is smooth, strongly cutinized, and contains no stomata. At the base of the ovule the abscission layer is marked by a distinct constriction (*fig. 10, a*). The opening of the micropyle is amber or brownish in color from the drying of the pollination drop which,

judging from the caliber of the micropyle, must be quite large, although it was not observed directly. Early in November the various tissues of the ovule are recognizable, and early in December the layers of the integument are almost as distinct as in the following March, although the cells of the stony layer have not begun to thicken, and microtome sections of the entire ovule can still be cut. The general topography of the ovule, as it appears later in December, is shown in *fig. 10*. In this figure the endosperm and fleshy tissues are dotted, the stony layer is more deeply shaded, and vascular bundles are represented by dark lines. The integument conists of three layers,

FIG. 7.—Ovule photographed from above; the eosin has spread and exaggerated the size of the outer bundles (*o*). ×2.

FIG. 8.—Inner vascular system of ovule, treated with eosin and photographed after the endosperm and part of the inner fleshy layer had been removed: *i*, bundles of inner vascular system; *m*, micropyle; *o*, bundle of outer vascular system; *p*, basal papilla; *s*, stony layer. ×2.

FIG. 9.—Transverse section of ovule treated with eosin: *e*, endosperm; *i*, bundle of inner vascular system; *n*, inner fleshy layer of integument and fused portion of nucellus; *o*, bundle of outer vascular system; *s*, stony layer. ×2.

an outer and an inner fleshy layer, with a stony layer between them. Only a small portion of the nucellus is free from the integument. The fleshy layers of the integument are comparatively simple in structure.

The outer fleshy layer.—The outer layer at the stage shown in *fig. 11* is not sharply marked off from the stony layer. These two layers

cannot be split apart, and even as late as May, when the stony layer has become very hard, the fleshy layer cannot be peeled off; it is only after it has become somewhat dry that it can be peeled off from the stony layer. However, the two layers easily separate as early as December if an ovule is cut in two and then allowed to decay for a few days in a damp atmosphere. The walls of the epidermal cells are considerably thickened and the outer surface is strongly cutinized. Most of the cells of the two layers just beneath the epidermis contain tannin, which occurs in only less abundance down to the level of the outer bundles. The cells containing tannin lie mostly in rows extending in the same general direction as the bundles. The large mucilage canals lie between the bundles and also have a general vertical course, but they branch and sometimes anastomose, so that some transverse sections of the canals are found even in longitudinal sections of the ovules. Beyond the zone of the bundles is a region of parenchyma cells (*fig. 11, p*).

The stony layer.—This layer, except at the deep pit which is occupied by the basal papilla, is thickest at the base and thinnest at the extreme apex. From the thin spot at the apex down to the lower part of the free portion of the nucellus it is thicker than throughout the middle two-thirds of the ovule. While the stony layer of the ripe seed is extremely hard, it is tougher and more elastic than the stony coat of most nuts, doubtless due to its complicated structure. The outer cells of the layer are small and isodiametric (*fig. 11, i*). In November they are not very sharply marked off from the inner cells of the outer fleshy layer; in fact, it is only after the walls of the stone cells have begun to thicken that the boundaries of the layers can be determined with accuracy. Beyond the small isodiametric cells is an irregular zone of cells elongated in a more or less longitudinal direction (*fig. 11, e*); then follows a zone of similar cells elongated transversely (*fig. 11, et*); and finally another zone of cells considerably elongated longitudinally (*fig. 11, i*). As growth continues, the small outer isodiametric cells simply increase in size and their walls became thickened. In the cells of the other three regions there is not only an increase in size and a thickening of the walls, but various displacements occur, so that the structure in March is much more complicated than in the preceding November. The elongating cells become

crowded and interlaced and sometimes even branch, thus giving rise to an extremely tenacious tissue,

The inner fleshy layer.—This layer contains the inner vascular system, but otherwise consists of rather uniform, slightly elongated parenchyma cells. The cells are smaller than those of the main body of the nucellus, but there is no definite boundary between them. The boundary between the inner fleshy layer and the stony layer is less indefinite, and as early as January the two layers may be peeled apart, though with a rather uneven break. In small November ovules the inner fleshy layer has about the same thickness as the stony layer, but from this time the fleshy layer grows more rapidly, and in early December ovules is much thicker than the stony layer (*fig. 10*). The rapidly growing endosperm then begins to encroach upon the adjacent cells, which we regard as nucellar tissue intimately united with the inner fleshy layer of the integument, although it must be admitted that the ontogeny shows no indication of such a union. The encroachment continues until in the ripe seed all the tissues between the endosperm and the stony layer is reduced to a thin dry membrane, which peels off easily and cleanly, and shows very clearly the distribution of the inner vascular system. The inner fleshy layer is to be regarded as belonging to the integument rather than to the nucellus, because it is continuous with the inner fleshy layer of the free portion of the integument; because the bundles which it contains often extend into the inner fleshy layer of the free portion of the integument but never into the free portion of the nucellus; because in half-ripe ovules the fused portion of the nucellus may be peeled away from the layer containing the inner bundles; and, still more important, because such a conclusion is warranted by a comparison of the cycad ovule with those of fossil gymnosperms.

The phylogeny of the three layers.—This is an interesting question. Has the single complex integument always been single, or does it represent two integuments which have become fused? This is a question to which no definite answer can be given, because the early development of the ovule has never received sufficiently careful study in any cycad. The early stages in the development of the integument are passed before the cone breaks through the scale leaves, and consequently no material is likely to be secured in greenhouses. In the

field, where material may be abundant, it would be necessary to cut out the growing points of many plants to secure a few ovulate cones. And even then, judging from a slight examination of the ovule of Ginkgo, the integument of which also has two fleshy layers with a stony layer between them, we should be likely to find the integument arising as a single undifferentiated tissue. A study of the integument of Dioon after its various tissues have become somewhat differentiated also fails to give any definite evidence as to its single or double nature.

I have been able to examine the integuments of several other cycads, but even where the differentiation between the layers is sharp, as in *Zamia integrifolia*, where the outer fleshy layer in both its cell-structure and cell-contents is sharply marked off from the stony layer, and where the differentiation between the stony layer and the inner fleshy layer is also rather distinct, there is no satisfactory evidence that the integument has ever been anything but a single structure. In Ceratozamia the layers are even less defined than in Dioon.

Judging from the literature of the subject, especially from the work of Miss STOPES (**16**), who has made the most thorough investigation, no study of the integument of living cycads can yield conclusive evidence as to its single or double nature. However, a comparison of the cycad ovule with that of the fossil Lagenostoma lends much strong support to the theory that the cycad integument is a double structure. OLIVER and SCOTT[13] suggest that the cupule of Lagenostoma is equivalent to the outer fleshy layer of the cycad integument, while the canopy of a Lagenostoma may have become simplified into the stony layer of the cycad seed. Miss STOPES' opinion is indicated by the title of her recent paper "On the double nature of the cycadean integument."[16] She believes that the plane of fusion between the two coats is either between the inner and outer portion of the stony layer or between the stony layer and the inner fleshy layer. The outer fleshy layer and the outer portion of the stony layer she believes to be too intimately connected to be separated morphologically. The structure of the Dioon integument, as it appears in sections, would seem to indicate that the plane of union has been between the inner and outer layers of the stone (*fig. 11, et, i*). The integument of Ceratozamia would bear a similar interpretation; but in *Zamia integrifolia* the outer fleshy layer is so sharply marked off from the

stony layer that one could easily regard the former as an adnate cupule equivalent to the free cupule of Lagenostoma. In spite of the close morphological continuity between the stone and the outer flesh during the early development of the integument of most cycads, the two layers separate readily at maturity. The carpels of syncarpous ovaries of angiosperms become so closely united that the planes of fusion seem completely obliterated and the tissues appear perfectly continuous, but at maturity the carpels separate along the original planes of union. Hence the close continuity of the tissues should not exclude the view that the outer fleshy layer of the cycad ovule represents a structure which has become adnate to the stony layer. On the other hand, the ready separation at maturity must not be urged too strongly as an argument in favor of a union at this plane, because at maturity the inner fleshy layer of all the cycads also separates just as readily from the stony layer, and the inner fleshy layer of the integument never separates at all from the lower portion of the nucellus. While it must be confessed that the cycadean integument itself offers no conclusive evidence of a double nature, we agree with Miss STOPES that it possibly represents a double structure. In regard to the plane of fusion we could not agree with her, but think it more probable that the union has taken place between the outer flesh and the stone.

The nucellus.—The nucellus is free from the integument only for a short distance, the free portion extending little beyond the top of the endosperm. The free surface is cutinized and the epidermal cells contain tannin. When very thick sections are cleared in xylol, the free portion is limited by a very conspicuous black line. The sharp beak closes and hardens immediately after pollination, imprisoning the pollen grains in the pollen chamber. After pollination the upper portion in the surface of the nucellus soon becomes marked by brown lines caused by the haustoria of the pollen, which never penetrate deeply, but lie just beneath the epidermis. In December a considerable mass of tissue separates the pollen chamber from the top of the endosperm, in which the archegonial chamber has not begun to form. Subsequent growth of the endosperm, together with extensive disorganization of nucellar tissue and also some enlargement of the pollen chamber itself, finally destroys all tissues between the pollen tubes and the archegonia. The mode of disorganization which results in the

formation of the pollen chamber is easily seen. The middle lamella softens and breaks down, thus setting free a few cells which are soon resorbed, and so forming the beginning of a pollen chamber. The subsequent encroachment of the disintegrating region is most rapid downward, but is also increasingly extensive at the periphery, so that the completed chamber is more or less funnel-shaped.

Below the free portion of the nucellus its most conspicuous feature is the thick jacket which surrounds the endosperm (*fig. 11, ej*). This jacket is sharply differentiated from the rest of the nucellus, and is so tenacious that it can be stripped off with forceps. The megaspore-membrane sometimes adheres to it, but more often clings to the endosperm. Although the material contained no early stages in the formation of the jacket, it is evident that it originates as a layer only one cell in thickness. Where cells of the layer do not divide periclinally, it remains only one cell thick; if there is a single periclinal division, the layer becomes two cells thick; while another division would make it three cells in thickness. In the archegonial region a considerable portion of the cells undergo one periclinal division; while in the chalazal region nearly all the cells show such a division, and a large number undergo a second periclinal division. On the sides there is a gradation between these two extremes. The less prominent and less complete jackets of the Coniferales have been described by THOMSON (**21**).

The cells of the jacket are very frequently binucleate and occasionally three nuclei are found. In comparison with the size of the cell these nuclei are rather large, but their chromatin content is very scanty and the nucleoli are small (*fig. 12*). It is not at all uncommon in these cells to find a complete chromatic spirem lying perfectly free in the cytoplasm, with no trace of a nuclear membrane or any achromatic structures (*fig. 13*). In such cases the nucleolus is always present and is somewhat larger than that of resting nuclei. The amount of chromatin in the spirem is vastly greater than in the resting nuclei. This unique condition does not lead to the formation of daughter nuclei or even to the reorganization of the single nucleus, and the significance of the phenomenon is not evident. The cells of the jacket at this time are centers of extreme metabolic activity, all the materials for the nutrition of the rapidly growing endosperm

passing through them, and in passing undergoing some change before reaching the endosperm. It may be that the peculiar nuclear condition is brought about by the extreme activity of the jacket cells.

The walls of the jacket cells are suberized and stain deeply with safranin, thus contrasting sharply with the cellulose walls on both sides of them. The most conspicuous cell contents are irregular granules and coarse strands, which stain so deeply with safranin that in a section of a November ovule the jacket appears as a bright red circle easily visible to the naked eye. The material of the granules appears very much like that of the outer part of the megaspore membrane, which THOMSON (**21**) found to be a suberized structure. He described similar granules in other cycads and found them to consist of amylodextrin, but in his form the amylodextrin disappeared before the stage represented in *fig. 13* was reached. (There are no pits in any of the cells of the jacket.) The irregular granules and strands adhere closely to the cell wall even after the cytoplasm has been drawn away by reagents, as shown in *figs. 12* and *13*. No granules or strands are found on the outer walls of the jacket cells or in the cells on either side of the jacket layer. The endosperm jacket differs decidedly from the archegonial jacket in having no pits in the walls; consequently, all substances must pass through the jacket by the usual method of transferring material from one cell to another.

The jacket is doubtless concerned with the nutrition of the structures within, like the jacket about the embryo sac of an angiosperm, the jacket about the seeds of gymnosperms, or the tapetum about the sporogenous cells of microsporangia. While the jacket in all these cases is concerned in nutrition, the mode of nutrition and the nature of it are different. It is most active while the endosperm is still spherical. As the endosperm elongates and approaches its full size, the jacket disintegrates, and at the stage shown in *fig. 10* it has broken up and only suberized fragments remain. As to its morphological nature, the jacket corresponds to the jacket or tapetum which surrounds the sporogenous tissue of microsporangia. In a microsporangium the jacket surrounds a large number of spores, while in the case under discussion it surrounds only one spore, which, at the stage shown in *fig. 10*, has developed an extensive prothallium. LANG (**12**), who secured early stages of this structure in Stangeria, describes an

extensive sporogenous tissue in the ovule, and believes that the outer portion of it gives rise to the tapetum, while all the tissue within except the functional megaspore disappears. Unfortunately, no such early stages were secured in Dioon, but since the jacket surrounding the microspores may originate from either sporogenous or sterile cells, there seems to be no objection to regarding the jacket surrounding the megaspore as equivalent to that surrounding the microspores, especially since homosporous forms, to which all heterosporous forms must ultimately trace their origin, have well-developed jackets.

Base of the ovule.—The lower limit of the ovule is marked superficially by a slight constriction as soon as the abscission region begins to develop (*fig. 10, a*). This region does not extend straight across, but is depressed in the center, with the depression away from the body of the ovule. When the ovule breaks off, the slight protuberance at the base is due to the shape of the abscission zone. This zone, about twelve cells in thickness, consists of nearly isodiametric cells, and thus contrasts rather sharply with the elongated cells above and below (*fig. 14*). Almost as soon as the zone can be distinguished, intercellular spaces appear, which increase in size until they sometimes become as large as the cells themselves, making them appear somewhat like the stellate cells of Typha, Scirpus, and similar forms. These cells are rich in starch. Above the abscission region the axis of the ovule extends upward and terminates in a conspicuous projection which occupies a deep pit in the stony layer. This projection, which was described as the *Einstülpung* by Miss STOPES (**15**), may be called the basal papilla (*fig. 10, p*). In the unripe seed the tissue of the basal papilla is noticeably tougher than that of other portions of the outer fleshy covering. In the ripe dry seed it looks for a time like a toothpick broken off in the stony layer, but it soon decays like the rest of the fleshy layer and its place is marked by a conspicuous pit about 2^{mm} in diameter and extending almost through the stone. Large elongated cells containing tannin are abundant on both sides of the abscission zone, but they do not extend into it. Several large mucilage ducts, which lie within the region occupied by the vascular bundles, reach almost to the top of the basal papilla. In the position and shape of the abscission layer and also in the appearance of the basal papilla, Dioon resembles Lagenostoma as described by OLIVER

and Scott (**13**) and illustrated in their *fig. 26*. The rest of the tissue on each side of the abscission zone consists of large, elongated cells, forming a loose parenchyma with irregular intercellular spaces like those of the abscission zone, except that the spaces are larger.

Vascular system of the ovule.—The distribution of the vascular system is easily traced by cutting off the petiole of the sporophyll under water and placing the cut end in an aqueous solution of eosin. The vessels become filled with the solution in a few minutes. For studying the course of the bundles in the ovule itself, it is better to cut the ovule just below the abscission layer. While the fluid does not always penetrate the ultimate tracheids, the course of the bundles is sharply marked, as may be seen in *figs. 7–9*, which are photographs of ovules treated in this way. For studying the transverse distribution of bundles, series may be obtained very rapidly by cutting sections of ovules treated with eosin and simply placing the sections in order upon a sheet of paper. The abundant mucilage causes them to adhere firmly, and the bundles appear as bright red dots.

A transverse section of the petiole of the megasporophyll near the axis of the cone shows about seven bundles, arranged in a straight line. Two of these bundles, one at each end of the row, are usually larger than the others, and develop into the vascular systems of the ovules. The other bundles branch repeatedly, so that in the widest part of the lamina of the sporophyll a transverse section shows about thirty bundles, still arranged in one straight line. The outer bundle forks once as it passes to the ovule, so that a transverse section just below the abscission line of the ovule shows two bundles. From each of these two bundles a branch passes toward the outer fleshy layer of the integument, and another branch toward the inner fleshy layer, thus giving rise to an outer and an inner vascular system with the stony layer lying between them (*fig. 10*).

The bundles which supply the outer fleshy layer of the integument branch several times before they reach the level of lower limit of the stony layer, but from this point they extend to the micropyle with no branching at all (*fig. 7*). The number of these bundles in vigorous ovules varies from 10 to 17, with 14 as the most frequent number; in abortive ovules 12 and 13 are the most frequent numbers. In every cone there are some abortive ovules, and it may be that an

inferior vascular supply has something to do with their failure to develop.

The bundles passing to the inner fleshy layer begin to branch a little higher up than do those of the outer layer. There is some branching in the basal papilla; after passing out to the inner fleshy layer there is repeated forking, as shown in *fig. 8*, so that a transverse section taken at the level of the archegonia shows 40 to 60 bundles. After passing from the stony layer, these bundles run in the outer part of the inner fleshy layer of the integument and extend up to the free portion of the nucellus. Occasionally a bundle continues in the inner fleshy layer beyond the beginning of the free portion of the nucellus, but in no case was a bundle found entering the nucellus itself. Occasionally a small bundle passes through the middle of the basal papilla, through the thin portion of the stony layer, and into the tissue at the base of the endosperm. The development of the bundles and the significance of the various details of the vascular system have not been attempted.

IV. THE FEMALE GAMETOPHYTE.

Stages showing the origin and early development of the female gametophyte were not secured. Since fruiting plants are comparatively rare, and since they cannot be distinguished from vegetative specimens until the cones are well started in their development, the earliest stages could be obtained only by a vandal-like mutilation of plants large enough to bear cones. The youngest ovules, collected early in November, were about 8^{mm} in length, and showed the archegonium initials. The gametophyte had become cellular throughout, no free nuclear portion remaining at the center. Only one small cone had ovules as young as this, the ovules in the cones of this date measuring about 1^{cm} in length and showing the central cell of the archegonium.

During November, December, and January the pressure exerted by the young gametophytes is remarkable. At a slight cut into the tough layer which is to become stony, a distinct snap can be heard, the cut gapes open, and the gametophyte protrudes. Later, when its cell walls have become firmer, the gametophyte retains its form when cuts are made into the stony layer. When an ovule in which the

gametophyte is still spherical is cut through the middle, the gametophyte, as seen with the naked eye, has a beautiful, radiating appearance, looking somewhat like a transverse section of a twig with very fine and numerous medullary rays. Until late in December the endosperm is almost transparent, so that the young archegonia are easily seen and counted, even when the endosperm is removed entire. Ordinary newspaper print can be read through a section of endosperm 4^{mm} in thickness. At this stage the endosperm tastes sweet on account of the abundant sugar, but as soon as starch begins to form it takes on a translucent white color, and as the starch becomes abundant an opaque white. Even before the starch begins to appear, the endosperm commences to elongate, and in February has reached a length of 15^{mm} and a breadth of 10^{mm}. In April, when the endosperm has reached its full size, it is about 25^{mm} in length and 17^{mm} in breadth.

Early in December there not only is no archegonial chamber, but the top of the gametophyte containing the young archegonia is even elevated (*fig. 15*). In February the tissue at the rim of the elevation begins to grow rapidly, while the growth of the elevation itself is checked; consequently, the elevation soon becomes the bottom of the archegonial chamber (*fig. 16*). At the time of fertilization the archegonial chamber has reached a depth of 1 to 1.5^{mm} (*fig. 17*).

The megaspore membrane.—Early in November, while the endosperm is still spherical, the megaspore membrane is well developed. In fresh material one might easily mistake the endosperm jacket for the membrane, but the jacket is much coarser and can be stripped off entire with forceps. The membrane is comparatively delicate, but pieces several millimeters in length may be stripped off with forceps. When the nucellus is removed, the portion of the membrane covering the archegonia usually adheres to the nucellus rather than to the endosperm. The membrane is thinner at the apex and base of the endosperm than on the sides. In young ovules, with the endosperm still spherical, the greatest thickness of the membrane which was measured was 3μ at the apex, 3.1μ at the base, and 4.5μ at the sides. The average at this stage is about 3μ at the base and apex, and 4.1μ at the sides. In ovules whose endosperm has reached a length of 1.5^{cm}, but in which the archegonial chamber has not yet begun to form, the membrane is rather uniformly about 5μ in thickness. In germinating

seeds in which the embryo is beginning to break the stony layer, the membrane reaches a thickness of 9–10μ, but the membrane is homogeneous, there being no differentiation into layers as in earlier stages.

The membrane clearly consists of two layers, which may be called the endospore and exospore (*figs. 12, 18*). The outer layer in young ovules is three or four times as thick as the inner, but in older ovules the difference is not so great. The inner layer seems perfectly homogeneous under the highest magnification. The outer layer, under moderate magnification, looks somewhat like the dense outer layer of the megaspore membrane of Marsilea; but with a Bausch and Lomb $\frac{1}{16}$ immersion and Zeiss oc. 12 the structure appears to be comparatively loose (*fig. 18*). The entire outer layer consists of club-shaped bodies. In other gymnosperms similar bodies have been described as prismatic, but in Dioon they really consist of a globular or ovoidal outer portion connected with the inner layer of the membrane by a stalk. In surface view these bodies are seen to be regularly arranged and to be quite uniform in size, the diameter of the head in November ovules being about 0.85 μ, and in the following February about 1μ. As will be seen from the figure, the stalks form a comparatively open structure between the heads and the endospore. This open region appears as a nearly black line when seen in sections more than 1 or 2μ in thickness, so that there seem to be three layers, the extra layer being only an optical effect caused by the stalk region.

The membrane covers the entire female gametophyte, but after the pollen chamber breaks through the base of the nucellus the portion of the membrane covering the archegonial chamber is ruptured, so that the two chambers form one continuous cavity. This cavity is moist, but is not filled with a liquid. Only a few chemical tests were made and the results agreed with those of THOMSON (**21**), who found the outer layer of the membrane to be suberized, while the inner layer iss uberized only where it is in contact with the outer layer. The inner portion of the inner layer, next the gametophyte, consists of cellulose. That the megaspore membrane is a vestige surviving from an ancestry which shed the megaspores seems too probable to be questioned. If it could be assumed that the ancestors of all the living cycads had megaspore membranes of equal thickness, and that in the surviving forms the membrane has been reduced uniformly in all genera, then

the living genus with the thinnest membrane could be regarded as the farthest removed from its ancestry, and the genus with the thickest membrane would represent most nearly the ancestral condition. In spite of the uncertainty of such suppositions, it seems reasonable to regard a thick membrane as a primitive character, and while not conclusive evidence in itself, it deserves to be considered with other features in any discussion of the phylogeny of the cycads.

It would be interesting to know the thickness of the megaspore membranes of the other cycads at various stages in the life history. THOMSON (**21**) found that in *Cycas revoluta* the membrane is slightly more than 5μ in thickness at the stage when cell division is beginning in the endosperm. At a somewhat later stage the same writer found the membrane of *Stangeria paradoxa* to be 4.5μ in thickness, and the same thickness is given for the membrane of mature seeds of *Zamia integrifolia;* while in *Ceratozamia longifolia* the membrane is 4.5μ in thickness at a stage when the archegonia have been formed. In *Dioon imbricatum*,[2] before the appearance of archegonia, the thickness was 3.8μ. I have measured the membrane in the above genera, except Stangeria, and find only such slight variations from THOMSON'S figures as may be accounted for by slight differences in stages of development. The extreme thickness of the membrane in the germinating seed of Dioon so much exceeds any of THOMSON'S measurements that it would be interesting to know whether the membranes of other gymnosperms increase so much in thickness when the seeds germinate. The megaspore membrane of Dioon is as thick as that of any cycad, and consequently, so far as this one character is concerned, Dioon is as primitive as any member of the group.

Development of the endosperm.—Early in November, about two months after pollination, the endosperm has become cellular throughout. If one might hazard a guess at previous stages, the November condition looks as if the free nuclear condition had been succeeded by the formation of very large cells which had been divided repeatedly. At a stage when the archegonium initial is first distinguishable, the peripheral cells of the endosperm about half way between the archegonial and chalazal regions present the appearance shown in *fig. 19*.

[2] This form is described by MIQUEL, in his *Prodromus Systematis Cycadearum* (1861), under *Dioon edule* Lindl., as *β imbricatum*, and is based upon leaf characters.

Except at its apex, the structure of the gametophyte is like that shown in this figure, a single layer of small peripheral cells being succeeded by layers of larger and larger cells. At the apex, where the archegonial initials are appearing, the single layer of small cells broadens rapidly into a lenticular group, from 7–10 cells in thickness. This group consists of about 200 cells, which are considerably smaller than those of the peripheral layer, as may be seen by comparing *figs. 19* and *23*, which are drawn to the same scale. The archegonium initials may be seen at the periphery of this group. At this stage, the cells of the gametophyte contain no starch or other food stuffs, the only visible contents being the nucleus, a scanty amount of cytoplasm, and the transparent cell sap.

Three weeks later, when the endosperm has reached a length of 15^{mm}, the cell contents seem to be just the same as before, no accumulation of foodstuffs being visible. Cell division has progressed rapidly, so that the row of four cells shown in *fig. 19, a*, is now represented by the two rows (sixteen cells) of *fig. 20, a*. Sections of the endosperm 4^{mm} thick, fixed in Flemming's solution early in December, appear somewhat darker in the region of the archegonia. In the chalazal region there is also a slightly darker color. Microtome sections show that the color is due in part to occasional grains of starch, but more particularly to small globules, probably oil, which stain black with osmic acid. The fact that the cells are considerably smaller in the archegonial region also makes the endosperm appear denser at this place. In the ripe seed the gametophyte shows considerable differentiation (*fig. 21*). The cells of the peripheral layer are small, rich in protoplasm, and contain numerous very minute starch grains. This layer contrasts sharply with the next, the cells of which are much larger and contain larger starch grains. This second layer is in turn fairly well marked off from the rest of the gametophyte, which consists of still larger cells densely packed with large starch grains. Numerous isolated cells containing tannin form a broad zone midway between the center and periphery of the endosperm. The tannin is much more abundant in the upper part of the gametophyte.

Development of archegonia.—Probably all of the superficial cells of the group at the apex of the endosperm are potentially archegonium initials. The number of archegonia varies from none at

all to ten. Several ovules at the period of fertilization showed no archegonial chamber and no trace of archegonia. Ten archegonia were observed in only a single instance, which was also exceptional in having two archegonial chambers. A single archegonium with a small archegonial chamber was observed in a few cases. Usually there are three, four, or five archegonia, with four as the most frequent number. An archegonium initial, which can be seen early in November, becomes distinguishable by its slightly greater size (*figs. 22, 23*). The division into a neck cell and central cell takes place early, probably in November (*fig. 24*). The neck cell divides almost immediately, forming the two-celled neck which is a constant feature in all cycads yet investigated. All December ovules not only showed the neck, but the neck had already divided into its two characteristic cells (*fig. 25*).

The central cell enlarges very rapidly, its scanty protoplasm forming a delicate layer pressed against the wall by the single large sap vacuole (*figs. 24, 25*). Even at the stage shown in *fig. 24*, the cells bordering upon the central cell are rather regularly arranged, and in *fig. 25* there seems to be a definite jacket. While this layer functions more or less in the nutrition of the central cell, the real archegonial jacket with its characteristic cell contents is formed later by both periclinal and anticlinal divisions in the jacket-like layer of *fig. 25*. The differentiation of the jacket is not so rapid at the apex of the central cell as at the sides and base. As soon as the jacket begins to be differentiated from the neighboring cells, the cytoplasm of the central cell increases rapidly, and soon the space which had been occupied by the single large vacuole is filled. The rapidly increasing cytoplasm shows a beautiful foam structure (*fig. 26*). At this stage there is no reticulum or even any fibers. The vacuoles of the central cell are much smaller at the apex and at its periphery than nearer the center. The walls of these vacuoles are themselves vacuoles, and there is a perfect gradation, so far as size and appearance is concerned, from the large vacuole near the center to the smallest vacuoles of the thin plates of cytoplasm which form the walls of the large vacuoles. Some of the walls of the larger vacuoles are shown in surface view in *fig. 26*, though most of the walls show only the edges, as in case of cell walls. The smallest of the vacuoles are small enough to come within BÜTSCHLI'S

limit for the size of meshes of genuine protoplasmic structure. The central cell of the archegonium is formed early in November, a little more than two months after pollination. The mitotic division which gives rise to the ventral canal nucleus and the nucleus of the egg takes place about the middle of the following May, so that the growth of the central cell and its nucleus extends over a period of six months. Throughout this entire period the growth is uniform, there being no cessation or retardation as would be the case in a colder climate.

The nucleus of the archegonium initial begins to enlarge very early, and is noticeably larger than the nuclei of the surrounding cells as soon as the initial itself becomes distinguishable (*fig. 22*); but while the growth of the nucleus continues steadily, it does not keep pace with the more rapid growth of the central cell. In March, after five months growth, the diameter of the nucleus has increased from 10μ to 70μ. The nucleolus, which at first was inconspicuous, has become prominent, and the chromatin granules are evenly distributed throughout the nucleus. At this time several large nucleolus-like bodies appear in the cytoplasm near the nucleus (*figs. 27, 29*). These bodies strongly resemble those which are found between the blepharoplast and nucleus in the body cell of Gingko, as described by HIRASE (**9**). At first they are solid and perfectly homogeneous; but as the nucleus of the central cell begins to divide, they become vacuolate (*fig. 29*), and before the mitosis has reached the metaphase they have broken up into innumerable small globules and granules (*fig. 30*). These bodies stain black with iron haematoxylin, and with the safranin gentian-violet combination they usually take the violet even when nucleoli are staining red with the safranin. Their exact nature and function was not determined, but they do not seem to differ, except in size, from the globules of nutritive material which are soon afterward brought into all parts of the egg by the haustoria.

The ventral canal nucleus.—While the growth of the nucleus of the central cell extends over a period of more than six months, its division, when once begun, is extremely rapid. The only figures found were in material sent from Xalapa on April 1, 1905, and fixed at Chicago on April 13 and 17. No figures were found in any of the numerous collections fixed in March. Some ovules fixed later than April 17, and even as late as May 13, showed the nucleus of the central cell

still undivided; but such ovules were from cones picked about the middle of March, and the ovules finally decayed without any division of this nucleus. The top of the typical archegonium in such material is shown in *fig. 28*, which is from a cone picked March 18, the material being fixed May 13, nearly two months later. The two neck cells are in perfect condition, but the central cell has begun to degenerate.

In normal material the spirem is broad but very tenuous in consistency. In the only cases noted it had evidently suffered from reagents (*fig. 29*). The shortening, condensation, and segmentation of the spirem were not observed, the next available stage being shown in *fig. 30*, where the segmentation into chromosomes has taken place. The splitting of the chromosome in the equatorial plate is shown in *fig. 31*. In this preparation the number of chromosomes was determined with reasonable though not absolute certainty to be twelve. In a late anaphase the chromosomes, while still retaining the U-shape, are becoming irregularly moniliform, looking as if they might break up into small pieces (*fig. 32*). Only a few stages in the formation of the spindle were observed. The first indication of it is a granular and fibrillar appearance which is more marked at the lower pole of the nucleus (*fig. 29*). There are no centrosomes, and in *fig.* 30 the poles are rather blunt. Fibers like the spindle fibers are abundant in the cytoplasm of the papillate projection in which the figure lies. In *fig. 31* the spindle is more sharply bipolar, and long mantle fibers are more conspicuous, although many of the spindle fibers are still continuous from pole to pole. *Fig. 32* shows no trace of the granules which mark the beginning of a cell plate, and later stages make it certain that no wall is formed between the daughter nuclei. The ventral canal nucleus remains free in the cytoplasm of the egg, as shown in *figs. 33–35*.

A ventral canal cell in cycads was first described by STRASBURGER (**18**) in 1876 for *Cycas sphaerica*. The next year WARMING (**23**) described one in *Ceratozamia robusta*, but soon concluded that he had been mistaken. TREUB (**22**) in 1884 failed to find any ventral canal cell in *Cycas circinalis*, and from that time it was generally believed that the cycads have no ventral cell. However, in 1898 IKENO (**10**) made an unmistakable demonstration of the critical mitosis in *Cycas revoluta*. At that time no ventral canal nucleus not separated from the egg

nucleus by a wall had yet been observed, and he merely followed current terminology. The term ventral canal nucleus was introduced by COKER (**3**) in 1902 to describe the condition in Podocarpus. IKENO's figures show that no wall is formed between the ventral canal nucleus and that of the egg. WEBBER (**24**) soon afterward reported that in Zamia "a small cell is cut off at the apex of the archegonium," but here too the language is unfortunate, for no cell is cut off, and the ventral canal nucleus remains in the general cytoplasm of the egg, as shown by COULTER and CHAMBERLAIN (**5**). I have recently made preparations of Encephalartos showing the same condition. It is probable that no ventral canal cell is cut off in any of the cycads, there being merely a nuclear division.

It cannot be doubted that this represents an advanced stage in the reduction of the archegonium. It offers no exception to the method by which the row of neck canal cells of the bryophytes and pteridophytes has been reduced. In these groups binucleate neck canal cells are frequent. This means that the formation of a cell wall has failed to follow the nuclear division. The next stage in reduction would be the suppression of the mitosis, and thus a diminution in the number of neck canal cells. In this way the neck canal cells gradually become reduced in number, some pteridophytes showing only a single one. In gymnosperms there is no neck canal cell at all, and the ventral canal cell is being eliminated by the same process. In some genera, like Pinus, the ventral canal cell is separated from the egg by a wall. In other genera the nuclear division takes place, sometimes with a series of granules on the spindle indicating a rudimentary cell plate, but no wall is formed. In Torreya it seems likely that even the ventral canal nucleus is suppressed. The absence of a ventral canal nucleus has been reported for several genera, but the evidence is not conclusive. Dioon, Zamia, Cycas, and Encephalartos still preserve the mitosis, although the wall is no longer formed. In this particular, although the oldest of living gymnosperms, the cycads do not show as primitive a condition as do Pinus and some other Coniferales.

After the mitosis described above, the ventral canal nucleus forms a membrane and may enlarge slightly, but it soon disorganizes, so that at the time of fertilization a scarcely recognizable vestige remains.

Typical views are shown in *figs. 33, 33a*, and *34*. Occasionally there is considerable enlargement (*fig. 35*), as is often the case in Pinus. I have a preparation of Encephalartos which not only shows a reorganization and enlargement of the ventral canal nucleus, but the nucleus has moved down from the papillate projection toward the egg, suggesting the possibility of fertilization of the egg by the ventral canal nucleus. In STRASBURGER'S account (**33**) of fertilization in *Picea vulgaris* and in COULTER'S account (**3**) in *Pinus Laricio*, describing fusing nuclei of equal size, a large ventral canal nucleus was doubtless mistaken for a male nucleus, but that these were actual cases of fertilization of the egg by its own ventral canal nucleus there can be but little doubt.

The egg and archegonial jacket.—The egg of Dioon is the largest yet known in plants. It is seldom less than 4^{mm} in length and often reaches a length of 5^{mm}; the largest egg measured was 6^{mm} in length. The actual size, just after the formation of the ventral canal nucleus, is shown in *fig. 17a*. The egg at the right in this figure is shown again in *fig. 17*, which is magnified six diameters so as to show the comparative size of the papilla, ventral canal nucleus, and egg nucleus. Before fertilization the egg nucleus becomes much larger and more deeply placed than is represented in this figure. In *fig. 15*, which shows the actual size of the central cell before the beginning of the archegonial chamber, and in *fig. 16*, showing the beginning of the archegonial chamber, the nucleus of the central cell is too small to be represented even by a single dot.

The nutrition of the egg is practically the nutrition of the central cell, for it reaches its mature character before the mitosis which separates the ventral canal nucleus from that of the egg. GOROSCHANKIN (**8**) in 1883 described a continuity of protoplasm between the jacket cells and egg of Ceratozamia. He believed that strands of protoplasm pass through sieve plates in the pits of the jacket. IKENO (**10**) in 1898 made a more detailed study of the growth of the egg in Cycas. He also described protoplasmic continuity between the jacket cells and the egg, and a passage of proteid materials from the jacket into the egg. Miss ISABEL SMITH (**14**), who studied the nutrition of the egg in *Zamia floridana*, found no connecting strands of protoplasm, but found projections, which she called haustoria,

extending from the egg into the jacket cells. In Dioon there are haustoria similar to those of Zamia. An examination of Ceratozamia, Cycas, and Encephalartos showed that they also have haustoria like those of Zamia and Dioon. The origin, development, and function of the haustoria, together with changes in the jacket cells of the surrounding tissue, would be a long problem, involving chemistry as well as morphology.

During the early stages of its growth (*figs. 24–26*) the central cell receives food material from the surrounding cells by the usual method of transferring substances from one cell to another. Up to the stage shown in *fig. 25*, the wall of the central cell is very thin, with no visible pits; but later the inner surface of the wall undergoes an extensive secondary thickening, interrupted only by the large pits which are such a conspicuous feature of cycad eggs. After the haustoria have become fully developed, their cytoplasm is in direct contact with that of the jacket cells, so that substances may pass from the jacket cells into the haustoria as readily as from one part of the jacket cell to another. A paper was received from Drs. STOPES and FUJII (**17**) just as this account is going to press, describing sieve plates as found by GOROSCHANKIN (**8**). Their figures are evidently drawn from young material, in which the haustoria have not reached their full development. That there is protoplasmic continuity here, as elsewhere, we do not doubt; but if the pit-closing membrane persists in stages like those shown in our *figs. 37–41*, it has escaped our observation. The haustoria project far into the cells of the jacket, and the mere thrust may have ruptured the membrane. At the stage shown in *fig. 36*, it is possible that the membrane may still be present; but in stages like *figs. 37–41* we could not find any membrane, even after a reexamination of our preparations. Consequently, we see no reason for modifying the following account, which was written before the paper by Drs. STOPES and FUJII was received.

The general method by which nutritive materials reach the egg is easily understood. The tissue of the female gametophyte is filled with starch and other food materials, which in a changed form pass into the archegonial jacket and thence into the egg. The cells of the archegonial jacket at certain times contain numerous starch grains, much smaller than those in the surrounding endosperm cells, but

the starch is soon dissolved. The change from starch to a soluble form doubtless takes place repeatedly, because the starch at various stages in the development of the archegonium is sometimes present and sometimes absent. The protoplasm of the jacket cells is abundant and their nuclei are much larger than those of the surrounding tissue. The changes taking place within these cells and nuclei resemble those which occur in glandular cells, there being a period of accumulation, followed by discharge, and then exhaustion, after which the processes are repeated. Glandular activity begins in December, as soon as the archegonial jacket becomes distinguishable. At this time the protoplasm of the central cell consists of a thin peripheral layer, and still thinner lamellae which divide the interior into vacuoles of various sizes, those near the center being the largest (*fig. 36*). The activity increases gradually up to the time of fertilization, about the first of May, and then diminishes. At the close of the period of free nuclear division in the proembryo, the jacket is not very vigorous, but there is still some activity. Toward the close of the intrasporal development of the proembryo, the jacket cells become weak in contents and begin to break down; and after the embryo has broken through the base of the egg and advanced 4 or 5^{mm} into the endosperm, the jacket is scarcely recognizable. During the period of repose or exhaustion (*fig. 36*) the protoplasm of the jacket cells is finely vacuolated. The nucleus has a somewhat homogeneous, finely granular structure, in which the chromatin is not conspicuous, although it can be seen that most of it is in the half of the nucleus nearest the egg. The protoplasm of the haustoria is evenly granular.

As activity begins (*fig. 37*), the protoplasm of the jacket cells becomes more coarsely vacuolated and food materials of various shapes and sizes appear within it. The nucleus is particularly active. The nucleolus becomes saturated with a substance which stains black with iron haematoxylin, and the chromatin first becomes conspicuous and then obscured by a substance which also stains black and may be the same as that in the nucleolus. Material passes from the nucleus into the cytoplasm and from the cytoplasm into the haustoria. Once within the haustoria, the food materials are already within the egg. In passing from the haustoria to the deeper portions of the egg, the materials break up into smaller and smaller granules

and globules, so that the periphery of the egg always contains the most conspicuous contribution from the jacket. The food materials never take the form of "proteid vacuoles" as in Pinus, and are never in danger of being mistaken for nuclear structures. The appearance of the jacket and the periphery after a discharge is almost completed is shown in *fig. 38*.

The three preceding illustrations (*figs. 36–38*) show the conditions in young eggs while the protoplasm is still quite scanty, most of the space being occupied by large vacuoles. Later stages, after the egg has become filled with protoplasm and the large vacuoles have disappeared, are shown in *figs. 39–41*. In *fig. 39* the ends of the haustoria are covered by a frothy substance, and in *fig. 40* it is seen that this substance is passing into the egg. A large vacuole marks the place which has just before been occupied by frothy substance. The nucleus, as is often the case, is concave on the side next the haustoria. A later stage is shown in *fig. 41*, which represents various kinds of food materials within the egg. Globules and droplets of various forms are most abundant, but crystalloids are not infrequent. The crystalloids are cubical or may approach the spherical form, but are never fusiform like those so characteristic of the egg of Zamia. Starch is sometimes present but generally absent.

The structure of the protoplasm of the egg undergoes great changes, especially during the last two months of its development. In December the protoplasm, containing one large vacuole, forms only a thin layer pressed against the periphery of the central cell (*fig. 25*). In March the protoplasm has increased greatly in quantity and numerous vacuoles have appeared (*figs. 36–38*). In these figures, and even in considerably later stages, the lines represent the edges of lamellae, and the appearance is a strong argument in favor of the foam or *Waben* theory of the structure of protoplasm. In later stages, however, the foam structure disappears, and the protoplasm seems to be almost entirely in the form of fibrillae (*fig. 41*). The change seems due in great part to the breaking down of lamellae, thus leaving fibrillae at the junctions.

Why the nucleus of the central cell remains at the apex for so many months, and why after the mitosis the egg nucleus moves down

while the ventral canal nucleus remains at the apex, are questions still unanswered. A moment's reflection will convince any one that neither temperature, light, nor gravity has any appreciable influence. The path by which nutrition reaches the egg may be an important factor, for the extensive vascular system of the ovule terminates near the top of the endosperm. On the other hand, the same position of the nucleus is found in gymnosperms whose ovules have scarcely any vascular tissue at all. Later, during the formation of the proembryo, the polarity is reversed, the nuclear activity being most vigorous at the base of the egg. At this time it is evident that nearly all the nutrition is coming from the endosperm at the base of the egg. As a working hypothesis it may be suggested that the early position of the nucleus at the apex, and also the reversal of the polarity, are due to chemotaxis, the source of nutrition being the controling factor.

The egg nucleus.—The egg nucleus of Dioon is the largest which has yet been found in plants. Its usual diameter is about 500 μ, but nuclei sometimes reach a diameter of 600 μ. Not infrequently the nucleus is elongated, and in such cases its bulk is likely to be greater than that of the usual spherical nucleus. One nucleus measured 1475 μ by 380 μ. In spite of the immense size of this nucleus, its structures could not be interpreted. Its chromatin content is shown at the lower end of the spindle in *fig. 32*. Stages immediately following this were not secured, and in stages like *figs. 34* and *35* the chromatin can no longer be identified. From this time to the entrance of the sperm into the egg, the internal structure of the nucleus is shown in *figs. 42–44*, which represent details as they appear under a magnification of 1300 diameters. The entire nucleus, drawn to this scale, would be more than 6^m in diameter! In all parts of the nucleus is found a delicate network of varying thickness, and upon it or imbedded in it are granules and globules of various sizes. The smallest granules, which are almost always associated with the network or clinging to the surface of larger globules, stain with gentian-violet. Most of the larger globules stain with safranin, so that the general tone of the network is red; but some stain with the gentian-violet and in others the two stains blend. There are always a few which do not stain at all. In most preparations the network stains very faintly or not at all. The nucleoli, which are large and vacuolated, can usually be distin-

guished from the globules represented in *figs. 42–44*. If any of these granules represent the chromatin, the individuality of the chromosomes would appear to be hopelessly lost. No satisfactory study of the living nucleus was made. A nucleolus is visible in living material, but otherwise the contents seem nearly homogeneous. There are few globules and the network could not be identified. It is possible that most of the globules and the network are coagulation artifacts due to fixing.

The development of the egg nucleus seems to be essentially the same in all gymnosperms. Among the investigators who have studied this nucleus in various gymnosperms may be mentioned STRASBURGER (**19**, **20**), IKENO (**10**), BLACKMAN (**1**), CHAMBERLAIN (**2**), FERGUSON (**7**), and LAND (**11**). BLACKMAN, CHAMBERLAIN, and FERGUSON attempted to follow the behavior of the chromatin, but the accounts are inadequate, there being stages in which all fail to identify convincingly the chromatin. Miss FERGUSON'S beautiful figures of Pinus show the familiar structures with great accuracy, but just what structures are chromatin is not clear. My own series of Pinus is less complete, and those of other writers are still more incomplete. In Dioon it is evident that this nucleus behaves much as in Pinus. The solution of the problem is not easily attained in cycads on account of the inaccessibility of material and the difficult sectioning. In Pinus, with its abundant material and easy technique, a close series in the development of the nucleus and in the formation of the spirem at the time of fertilization would probably lead to an understanding of the egg nucleus of the whole group.

SUMMARY.

1. Dioon occurs in abundance at Chavarrillo, Mexico.

2. It is probable that plants often reach an age of more than one thousand years.

3. The ovulate strobilus is more like the loose ovulate strobilus of Cycas than the compact ovulate strobili of the other genera.

4. The megasporophylls are more leaf-like than those of any other genus of cycads except Cycas.

5. The integument consists of three layers: an outer and an inner fleshy layer, with a stony layer between them. The integu-

ment is probably double in nature, the outer fleshy layer representing the outer integument.

6. Only a small portion of the nucellus is free from the integument.

7. One vascular bundle passes from the sporophyll toward each ovule. Before entering the ovule the bundle forks, one branch forming the continuously branching system of the inner fleshy layer of the integument, and the other forming the slightly branched system of the outer fleshy layer.

8. The megaspore membrane varies in thickness from 3–4.5 μ in young ovules to 9–10 μ in mature seeds.

9. The number of archegonia varies from one to ten, with four and five as the most frequent numbers. The archegonium initial appears in October; the division into neck and central cell takes place almost immediately after; the mitosis which forms the ventral canal nucleus and egg nucleus takes place during the next May.

10. The central cell and egg during earlier stages receive food substances by the ordinary method of nutrition, but later receive food material through haustorial projections from the egg which are in direct contact with the cytoplasm of the jacket cells.

11. There are twelve chromosomes in the egg nucleus, which is the largest one yet known in plants.

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LITERATURE CITED.

1. BLACKMAN, V. H., On the cytological features of fertilization and related features in *Pinus sylvestris* L. Phil. Trans. Roy. Soc. London B. **190**: 395–426. *pls. 12–14.* 1898.

2. CHAMBERLAIN, C. J., Oogenesis in *Pinus Laricio*. BOT. GAZETTE **27**: 268–280. *pls. 4–6.* 1899.

3. COKER, W. C., Notes on the gametophytes and embryo of Podocarpus. BOT. GAZETTE **33**:89–107. *pls. 5–8.* 1902.

4. COULTER, J. M., Notes on the fertilization and embryogeny of Conifers. BOT. GAZETTE **23**:40–43. *pl. 6.* 1897.

5. COULTER, J. M., and CHAMBERLAIN, C. J., The embryogeny of Zamia. BOT. GAZETTE **35**:184–194. *pls. 6–8.* 1903.

6. COULTER, J. M., and LAND, W. J. G., The gametophytes and embryo of *Torreya taxifolia*. BOT. GAZETTE **39**:161–178. *pls. 1–3.* 1905.

7. FERGUSON, MARGARET C., Contributions to the knowledge of the life history of Pinus. Proc. Wash. Acad. Sci. **6**:1–202. *pls. 2–24.* 1904.

8. GOROSCHANKIN, J., Zur Kenntniss d. Corpuscula bei den Gymnospermen. Bot. Zeit. **41**:825–831. *pl. 70.* 1883.

9. HIRASE, S., Études sur la fécondation et l'embryogénie du *Ginkgo biloba*. Jour. Imp. Coll. Sci. Tokyo **8**:307–322. *pls. 31–32.* 1895.

10. IKENO, S., Untersuchungen über die Entwickelung der Geschlectsorgane und der Vorgang der Befruchtung bei *Cycas revoluta*. Jahrb. Wiss. Bot **32**:557–602. *pls. 8–10.* 1898.

11. LAND, W. J. G., Spermatogenesis and oogenesis in *Ephedra trifurca*. BOT GAZETTE **38**:1–18. *pls. 1–5.* 1904.

12. LANG, W. H., Studies in the development and morphology of cycadean sporangia. II. The ovule of *Stangeria paradoxa*. Annals of Botany **14**: 281–306. *pls. 17–18.* 1900.

13. OLIVER, F. W., and SCOTT, D. H., On the structure of the paleozoic seed, *Lagenostoma Lomaxi*. Phil. Trans. Roy. Soc. London B. **197**:193–247. *pls. 4–10.* 1904.

14. SMITH, ISABEL, The nutrition of the egg in Zamia. BOT. GAZETTE **37**: 346–352. 1903.

15. STOPES, M. C., Beiträge zur Kenntniss der Fortpflanzungsorgane der Cycadeen. Flora **93**:435–482. 1904.

16. ———, On the double nature of the cycadean integument. Annals of Botany **19**:561–566. 1905.

17. STOPES, M. C., and FUJII, K., The nutritive relations of the surrounding tissues of the archegonia in gymnosperms. Beih. Bot. Centralbl. **20**: 1–24. *pl. 1.* 1906.

18. STRASBURGER, E., Ueber Zellbildung und Zelltheilung. Leipzig. 1876.

19. ———Befruchtung und Zelltheilung. Jena. 1878.

20. ———, Uber den Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. Hist. Beitr. **4**:1–158. *pls. 1–3.* 1892.

21. THOMSON, R. B., The megaspore membrane of the gymnosperms. Univ. Toronto Studies, Biol. Ser. no. 4. 85–145. *pls. 1–5.* 1905.

22. TREUB, M., Recherches sur les Cycadées. 3. Embryogénie du *Cycas circinalis*. Ann. Jard. Buitenzorg **4**:1–11. *pls. 1–3.* 1884.

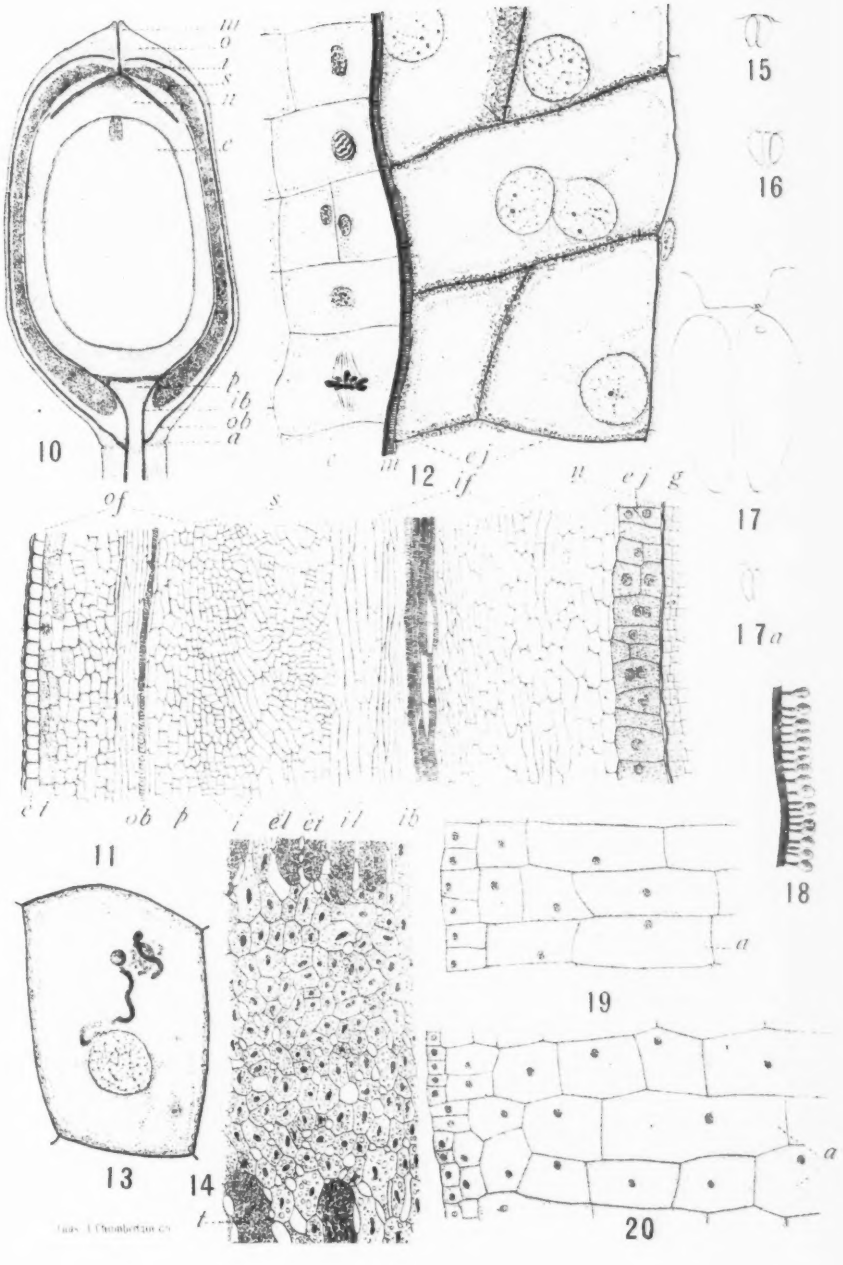
23. WARMING, E., Recherches et remarques sur les Cycadées. Oversigter K. D. Vidensk. Selsk. Forh. 1877.

24. WEBBER, H. J., Notes on the fecundation of Zamia and the pollen tube apparatus of Gingko. BOT. GAZETTE **24**:225–235. *pl. 10.* 1897.

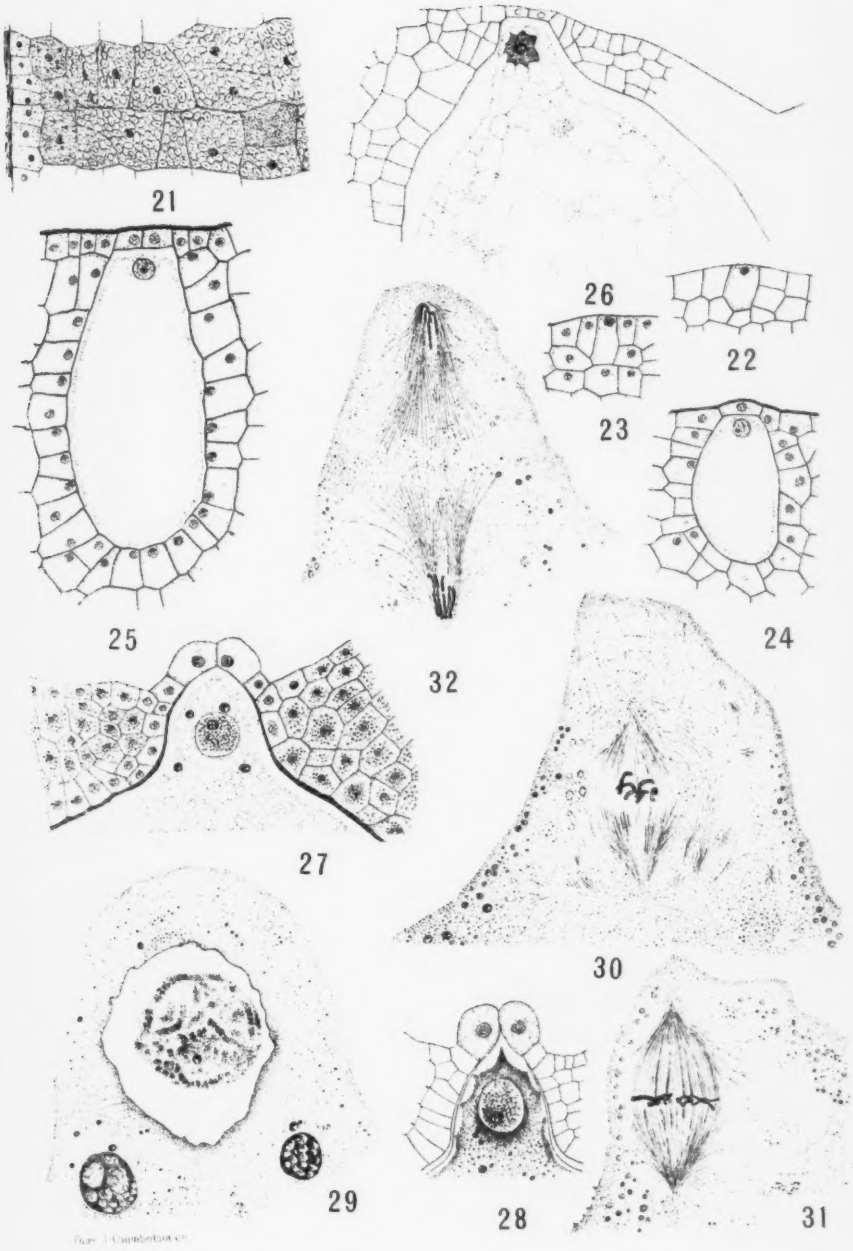
EXPLANATION OF PLATES XIII–XV.

(FIGS. 1–9 are text cuts.)

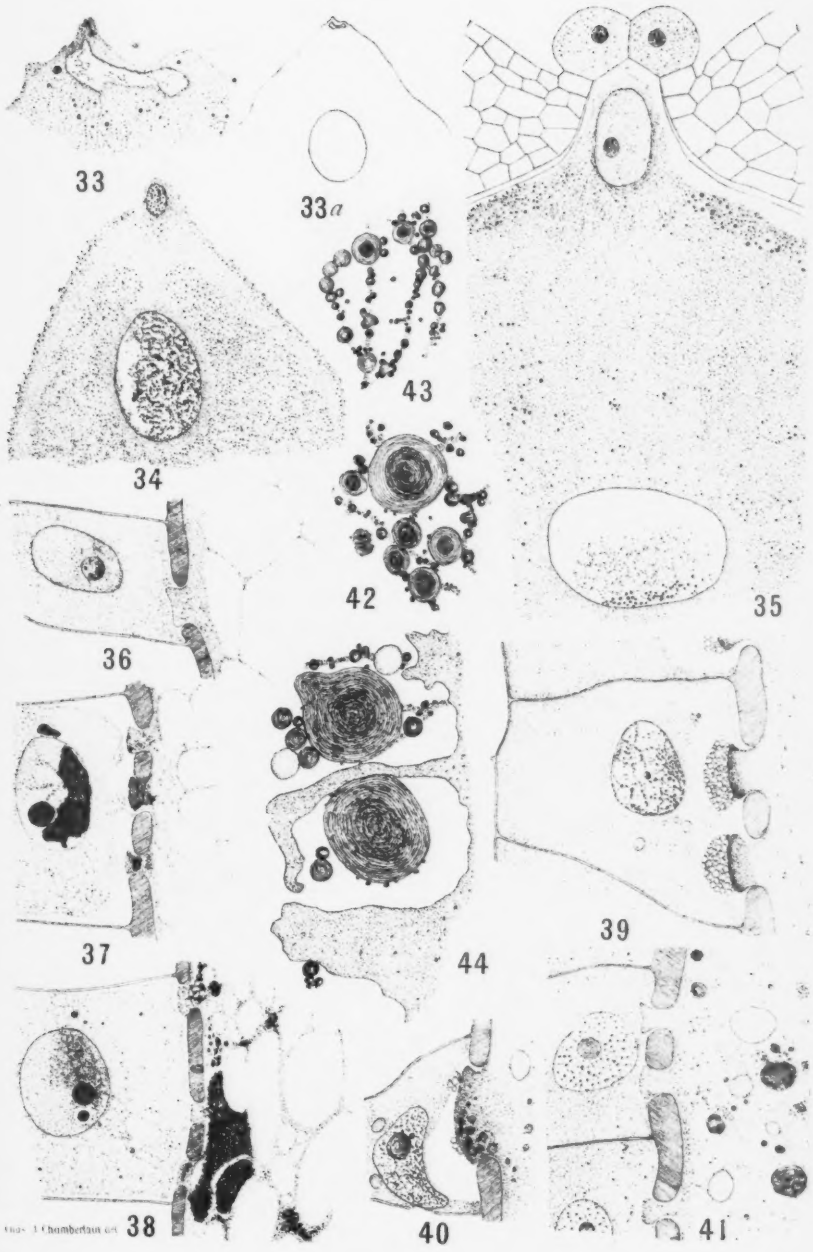
FIG. 10. Longitudinal section of ovule: *a*, abscission layer; *e*, endosperm; *i*, inner fleshy layer of integument; *ib*, bundle of inner vascular system; *m*, micropyle; *n*, free portion of nucellus; *o*, outer fleshy layer of integument; *ob*, bundle of outer fleshy layer; *p*, basal papilla; *s*, stony layer of integument. ×2.



CHAMBERLAIN on DIOON



CHAMBERLAIN on DIOON



CHAMBERLAIN on DIOON

FIG. 11. Longitudinal section through integument and adnate portion of nucellus: *e*, epidermis; *el*, longitudinally elongated cells of stony layer; *et*, transversely elongated cells of stony layer; *ej*, endosperm jacket; *g*, endosperm; *i*, isodiametric cells of stony layer; *ib*, bundle of inner vascular system; *if*, inner fleshy layer of integument; *il*, inner layer of longitudinally elongated cells of stony layer; *n*, adnate portion of nucellus; *ob*, bundle of outer vascular system; *of*, outer fleshy layer of integument; *p*, parenchyma cells of outer fleshy layer; *s*, stony layer; *t*, cells containing tannin. ×2.

FIG. 12. Section showing endosperm (*e*), megaspore membrane (*m*), and endosperm jacket (*ej*). ×350.

FIG. 13. Cell of endosperm jacket showing one normal nucleus and a free spirem of another nucleus. ×350.

FIG. 14. Longitudinal section through abscission layer; *t*, cells containing tannin. ×112.

FIG. 15. Section of archegonia early in December, showing endosperm somewhat raised over the archegonia. Natural size.

FIG. 16. Section of archegonia showing beginning of archegonial chamber. Natural size.

FIG. 17. Section of archegonia just after formation of ventral canal nucleus. ×5.

FIG. 17*a*. The same, natural size.

FIG. 18. Transverse section of megaspore membrane. ×900.

FIG. 19. Section of lateral endosperm at a period when archegonium initials are forming at the apex; 280 cells in entire periphery of section. ×88.

FIG. 20. Similar section at a later stage, 780 cells in periphery; the two rows of cells marked *a* correspond to the single row *a* of *fig. 19*. ×88.

FIG. 21. Peripheral portion of endosperm at period of germination. ×88.

FIGS. 22–23. Archegonium initials. ×88.

FIG. 24. Archegonium, Dec. 21. ×88.

FIG. 25. Archegonium early in January. ×88.

FIG. 26. Upper portion of archegonium. ×88.

FIG. 27. Upper portion of archegonium, March 7. ×88.

FIG. 28. Upper portion of archegonium, May 13, over two months after cone was picked. ×88.

FIG. 29. Nucleus of central cell and nucleolus-like bodies in the cytoplasm. ×350.

FIGS. 30–32. Stages in the division of the nucleus of the central cell to form the ventral canal nucleus and egg nucleus. ×350.

FIG. 33. Ventral canal nucleus. ×350.

FIG. 33*a*. Upper portion of egg, showing ventral canal nucleus and egg nucleus. ×22.

FIG. 34. Ventral canal nucleus and egg nucleus, April. ×41.

FIG. 35. Neck, ventral canal nucleus, and egg nucleus. ×41.

FIG. 36. Portion of jacket cell and egg, showing haustorium, March 9. ×800.

FIG. 37. Jacket and haustoria, March. ×800.

FIG. 38. Porton of egg and jacket cell just after a discharge. ×800.

FIG. 39. Jacket cell and egg showing accumulation of globules over the haustoria. ×800.

FIG. 40. Similar haustorium with the globules passing into it. ×800.

FIG. 41. Jacket cells and haustoria when the egg is nearly ready for fertilization. ×800.

FIGS. 42–43. Details of structure of the egg nucleus. ×1300.

FIG. 44. Small portion of the egg nucleus showing the very irregular outline of the nuclear membrane. ×1300.

TEMPERATURE AND TOXIC ACTION.

CHARLES BROOKS.

(WITH THIRTY-THREE CHARTS)

THE purpose of these experiments, the results of which are presented in this paper, was to determine what might be the modifying effect of temperature on the toxic properties of certain chemicals as shown by the effect of these substances on germination and growth in certain fungi. Since chemical processes as well as plant activities are influenced by temperature, it was thought that additional knowledge in regard to the nature of the physiological action of poisons might be obtained by comparing their effects at the optimum temperature for germination and growth of the plant with results secured under otherwise similar conditions, but at temperatures below and above that which is most favorable for the development of the particular plant.

So far as the writer has been able to learn, the problem of toxic action has never been carefully investigated from this standpoint. It is well known that temperature is an important factor in the processes of plant and animal life, and that changes in temperature may often serve as a stimulus to reproduction, germination, and development. It has also been shown that the response of an organism to certain stimuli may vary with the temperature, and some data have been reported which indicate that this is true when the stimulus is of a chemical nature. THIELE (1) found that the maximum temperature for the growth of *Penicillium glaucum* on grape sugar lies at 31° C., on glycerin at 36° C., on salts of formic acid at 35° C. NÄGELI (2) reported that bacteria were killed at 30 or 110° C. according to the character of the nutrient medium, but his conclusions seem to be based upon results obtained from impure cultures. HEIDER (3) found that the toxic action of certain chemicals upon the spores of *Bacillus anthracis* increases with a rise of temperature. PASTEUR (4) found that bacteria were more resistant to heat in alkaline than in acid milk; but COHN (5) and BREFELD (6) observed no such increased resistance in alkaline solutions. RICHET (7) has reported that with

various poisons the toxic dose diminishes in amount with the elevation of the temperature of the body. MATHEWS (8) found that a small rise in room temperature increased the toxic action of certain salts upon the eggs of the fish *Fundulus heteroclitus*, but no data in regard to the extent of the injury were reported.

Considerable work has been done in recent years on the effect of toxic agents upon the germination and development of fungi. CLARK (9) determined the concentration of various chemical solutions necessary to produce injury, inhibition, and death in certain fungi. He found that a solution of $n/4$ HNO_3 killed the spores of *Sterigmatocystis nigra* within forty-eight hours, that $n/8$ to $n/16$ solutions of the same acid produced total inhibition of the spores, and that $n/32$ gave great injury to the fungus. *Botrytis vulgaris* spores were killed by $n/16$, and the plant was greatly injured by $n/32$ HNO_3. With *Penicillium glaucum*, $n/4$ HNO_3 killed the spores, $n/8$ and $n/16$ totally inhibited germination, and $n/32$ gave decided injury. H_2SO_4 gave similar results, but a concentration of $n/2$ was required to kill the spores of Sterigmatocystis and Penicillium. With $CuSO_4$, $n/4$ killed the spores of Sterigmatocystis, $n/8$ to $n/16$ gave total inhibition, and $n/32$ to $n/64$ caused decided injury. Botrytis spores were killed by $n/16$ $CuSO_4$, inhibited by $n/32$, and the plant greatly injured by $n/64$. The spores of Penicillium were killed by $2n$ and inhibited by n to $n/64$, while decided injury resulted from $n/128$. DUGGAR (10) has reported upon special factors that influence the germination of fungous spores, and Miss FERGUSON (11) has given some of the conditions for germination in various basidiomycetous fungi. These recent papers have only an indirect bearing upon the work that follows, but have been very useful in the suggestion of methods for the solution of the problem.

METHODS.

The effect of the various toxic solutions at the different temperatures was observed by means of the ordinary Van Tieghem cells. The manner of constructing and the method of using these have been fully described by CLARK (9) and DUGGAR (10).

These cells were never used a second time without being taken apart and thoroughly cleaned. In cleaning, the cells were boiled for twenty or thirty minutes, first in an alkali, then in an acid, and

finally in distilled water. They were dried from alcohol and made up in the usual manner. The covers were treated as the cells, except that in each instance they were heated for a longer time, and that they were given one or two final boilings in redistilled water. All flasks, vials, etc., used in these experiments were cleaned with alkali, acid, and distilled water by boiling, as described for the cells.

As a culture medium several vegetable decoctions were tried. It was found that the five fungi used in these experiments grew well upon decoctions made from onions, beets, tomatoes, grapes, parsnips, beans, mushrooms, and sugar beets. Several series of experiments were made with tomato decoction as a medium, but it was found that a sugar beet solution gave less precipitate in the presence of $CuSO_4$ and was in general more satisfactory for the work. In all the experiments reported in this paper beet decoction was used as the nutrient medium. In making the infusion 600 grams of beets were used for every liter of water. At the time of using, the decoction was diluted, by the addition of the toxic solution and water, to one-half of its former nutrient value.

The toxic agents used were HNO_3, H_2SO_4, and $CuSO_4+5H_2O$. The chemicals were of the highest quality that could be obtained and the acid solutions were standardized before using. It is a well-known fact that strong concentrations of $CuSO_4$ precipitate proteids. In solutions at ordinary temperatures in which both are present, this precipitation continues for a long time, thus continually changing the nature of the liquid. Therefore, as it was necessary to make experiments at considerable intervals of time, the toxic agent was not added to the beet decoction until the time of its use in cultures. Stock solutions of the chemicals were made in water that had been carefully redistilled from glass in the presence of an oxidizing agent. Normal or one-half normal solutions were made and these stored in flasks provided with closely fitting rubber stoppers. By means of a series of graduated vials, these stock solutions were diluted and mixed with the beet decoction at the time of using.

The fungi used were *Botrytis vulgaris*, *Monilia fructigena*, *Sterigmatocystis nigra*, *Mucor Mucedo*, and *Penicillium glaucum*. The first two may be and usually are parasitic, and have an optimum temperature that is comparatively low; the last three are saprophytic

and grow well at temperatures considerably above the optimum for the first two. It was thought by this selection to obtain more interesting results than with forms more closely related physiologically. Only pure cultures were used. In the test tube cultures from which the spores were obtained for use, the fungi were grown upon cylinders of potato or beet. In either case the liquid in the tubes was a decoction of sugar beets. Other nutrient substances were tried for the test tube cultures, but these usually produced modifications in the growth of the fungi and it was not found advisable to use spores produced on different media in the course of a series of experiments, the results of which were to be compared. The spores used were always taken from cultures that were twelve to sixteen days old. The desired temperatures were secured by means of incubators and a refrigerator.

CLARK has pointed out certain sources of error for Van Tieghem cell cultures exposed to ordinary temperatures; but the placing of cells, made up under ordinary laboratory conditions, at temperatures ranging from 5° to 30° C., gave additional opportunity for error. The cells were not entirely closed until they had been left for several minutes in the temperature at which they were to remain. This gave opportunity for adjustment of air pressure in the cell, but it did not in all cases prevent the condensation of water vapor upon the cover glass. The small drops of water thus formed not only increased the evaporating surface but also modified the vapor pressure in the cell. The small water drops adjacent to the hanging ones of the nutrient solution seemed to sometimes unite with them, thus changing both their size and concentration. When the cultures were made in the dry air of a furnace heated room no difficulty was experienced, but cells made upon sultry days, or when the air of the culture room was humid from any cause, gave a visible condensation when placed at low temperatures. Even with the greatest precaution this difficulty was not entirely overcome.

It was found difficult to examine the cultures placed at various temperatures without interfering with the structure and condition of the cells. Examinations were made at temperatures as near as possible to those at which the fungi were growing, and results obtained from damaged cells were rejected. All cultures were observed every

twenty-four hours and notes taken of percentage of germination, length of germ tube, fruiting, and any peculiarities in germination or development. More frequent observations would have been of interest, but they were not made on account of the increased source of error that would have been thus introduced. Any sources of error that were not otherwise provided for were guarded against by always making duplicate cultures. The experiments with the three chemicals were always made at different times, and as control cultures were made in every case, the growth of each fungus in a nutrient medium at a particular temperature was tested six times.

The vitality of spores that had been subjected to the action of an inhibiting toxic agent was tested by transfer to a nutrient non-toxic medium. An attempt was made to accomplish this transfer by removing the drop of the toxic solution with sterilized filter paper and replacing it by a drop of beet decoction. This method left some part of the former solution as well as any precipitate that had been formed adhering to the cover glass, and was therefore adandoned. All transfers that are concerned in the following data were made by means of a sterilized platinum needle. The spores were in every case transferred to a drop of beet decoction on a clean cover glass. The medium used in the bottom of the cells was in this, as well as in all other cases, the same as that of the hanging drop. It is quite evident that the above method of transferring did not prevent a small amount of the toxic solution being carried into the new drop by the spores and the needle, but the results obtained indicated that this small per cent. of the toxic agent either served as a very slight stimulus to germination and growth or exerted no appreciable influence.

Early in the work it was seen that results obtained from the exposure of the fungous spores to the toxic agent must be considered entirely apart from the data secured in cases where the mycelium was acted upon by the toxic solution. Therefore, when a particular toxic solution gave no germination at one temperature, but did at others, the ungerminated spores were in no case transferred; *i. e.*, transfers were made only with those solutions that gave no germination even at the optimum temperature at the end of the given time.

By a series of preliminary experiments strengths of toxic solution

and time of exposure were determined, such as would give the greatest contrast in the results obtained at the various temperatures.

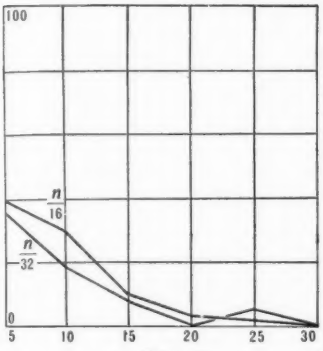
DATA AND DISCUSSION.

In order to put the results obtained in a form as concise as possible, charts 1*a*–12*c* have been prepared, and the greater part of the data obtained is expressed in these by means of curves.

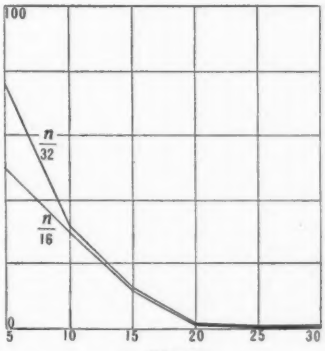
In charts 1*a* to 10*c* inclusive, the abscissae indicate the temperatures at which the fungus was kept in culture, and the ordinates show the per cent. of germination at these temperatures. All the points indicating per cent. of germination at the various temperatures for a particular toxic solution are joined by solid or broken lines; the strength of the toxic solution used is shown by the fraction placed on or near the particular curve. For a further illustration of the meaning of these charts, the curves in chart 2*a* may be considered. These represent the data secured by using $CuSO_4$ with Monilia. With an $n/16$ solution no germination was obtained at 25° and 30°; but at 15°, 12 per cent. of the spores germinated; at 10°, 30 per cent.; and at 5°, 49 per cent.

In charts 1*a* to 5*c* inclusive the results were obtained by exposing the spores for twenty-four hours at the various temperatures in the toxic solution indicated and then transferring them as previously described. The charts are based entirely upon the data secured on the first and second days after transferring. The solid lines indicate the total germination at the end of the second day. The broken lines show the per cent. of germination twenty-four hours after transferring. Where the record of germination was the same for the two days only the solid line is used. It will be noticed that only in a very few instances did spores germinate on the second day.

It is readily seen that in most cases the deleterious action of the toxic agents increased very rapidly with the rise in temperature. A comparison of the charts for the various fungi indicates that there are some differences in reactions worthy of special note. Thus, there is a marked drop between 5° and 10° in the germination curves for Botrytis and Monilia, but for no other fungus. With Penicillium the fall comes either between 10° and 15° or between 15° and 20°, while with Mucor and Sterigmatocystis the downward curves begin

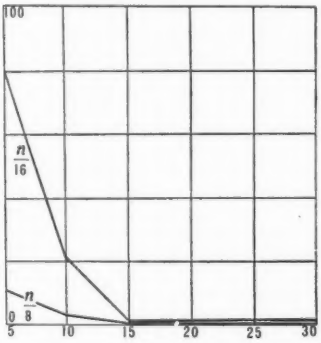


1*a*, Botrytis.

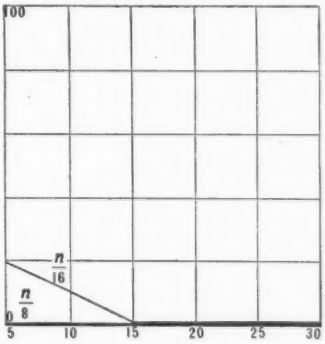


2*a*, Monilia.

$CuSO_4$.

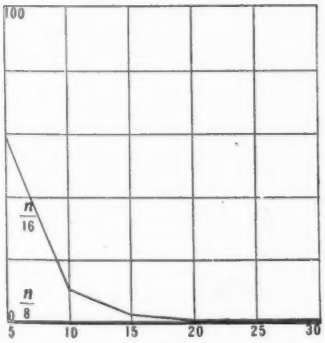


1*b*, Botrytis.

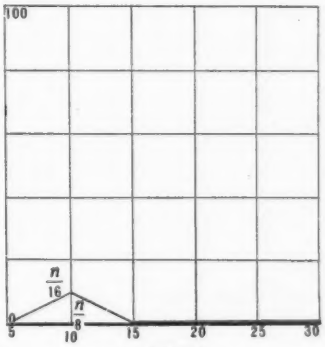


2*b*, Monilia.

H_2SO_4.

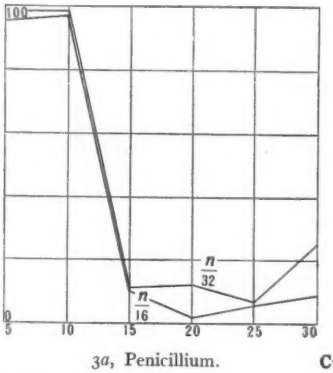


1*c*, Botrytis.



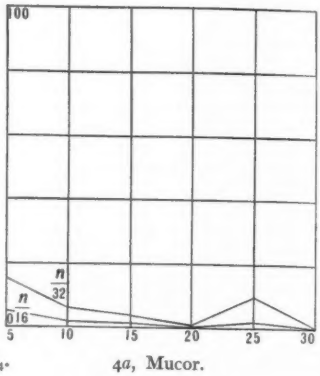
2*c*, Monilia.

HNO_3.

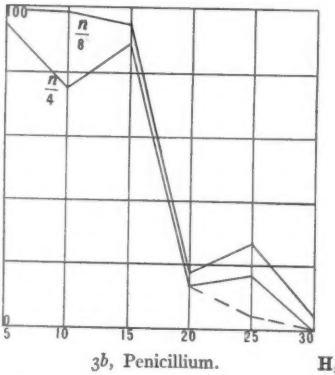


3*a*, Penicillium.

$CuSO_4$.

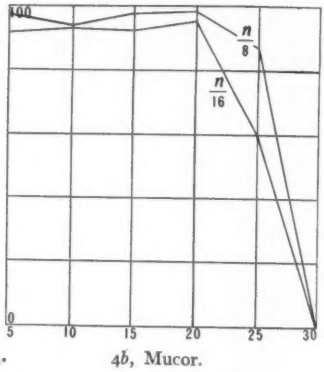


4*a*, Mucor.

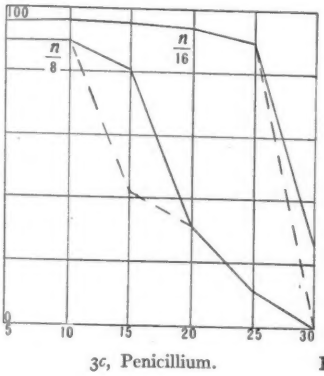


3*b*, Penicillium.

H_2SO_4.

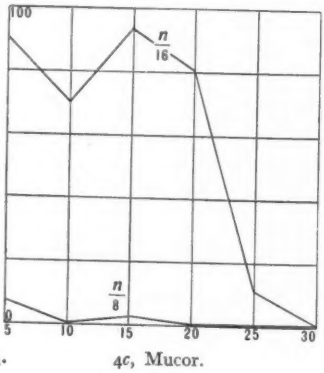


4*b*, Mucor.

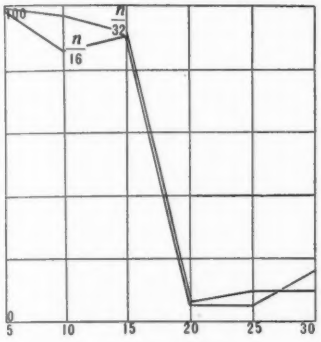


3*c*, Penicillium.

HNO_3.

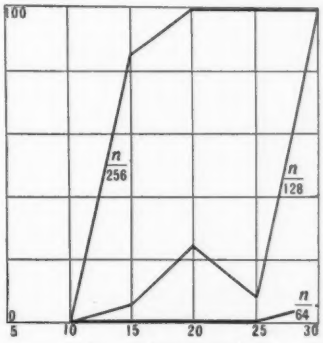


4*c*, Mucor.

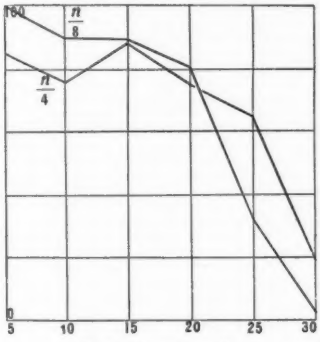


5*a*, Sterigmatocystis.

$CuSO_4$.

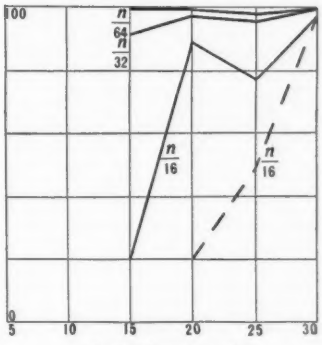


6*a*, Sterigmatocystis.

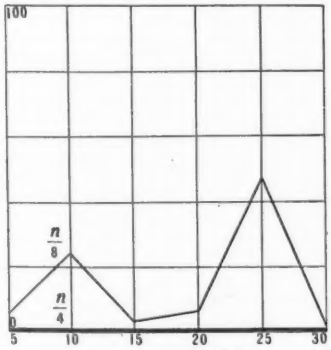


5*b*, Sterigmatocystis.

H_2SO_4.

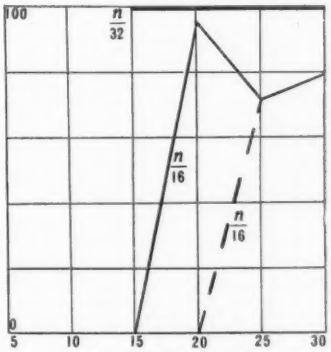


6*b*, Sterigmatocystis.

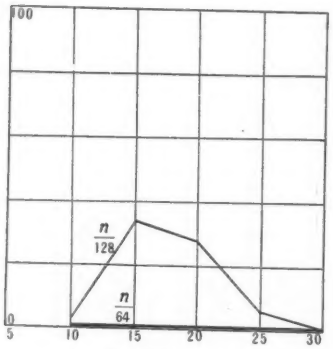


5*c*, Sterigmatocystis.

HNO_3.

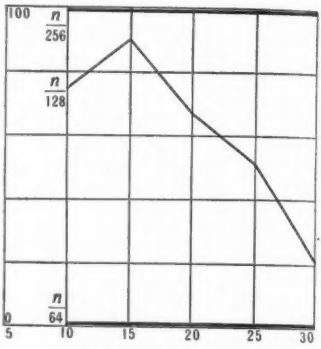


6*c*, Sterigmatocystis.

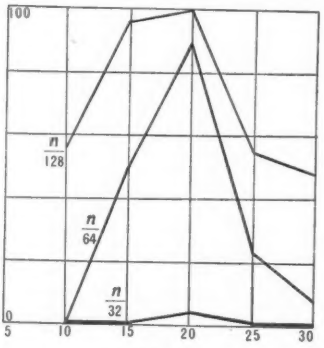


7*a*, Mucor.

$CuSO_4$.

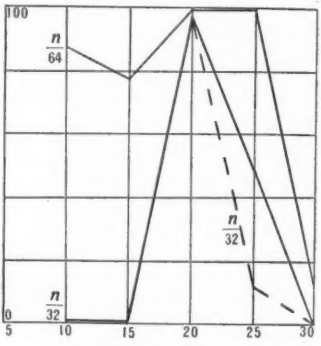


8*a*, Penicillium.

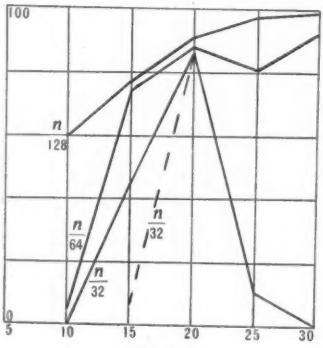


7*b*, Mucor.

H_2SO_4.

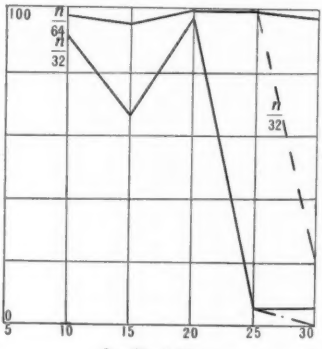


8*b*, Penicillium.

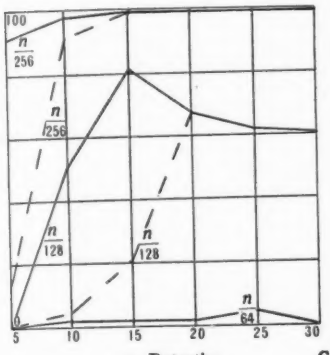


7*c*, Mucor.

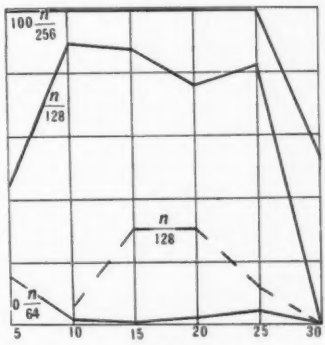
HNO_3.



8*c*, Penicillium.

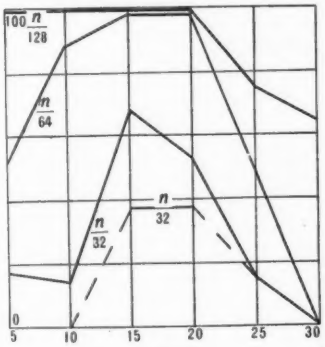


9*a*, Botrytis.

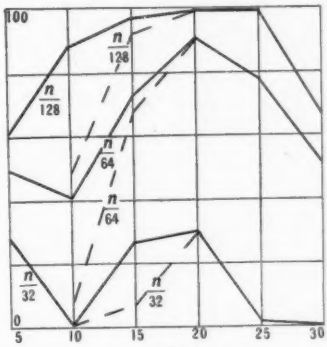


10*a*, Monilia.

$CuSO_4$.

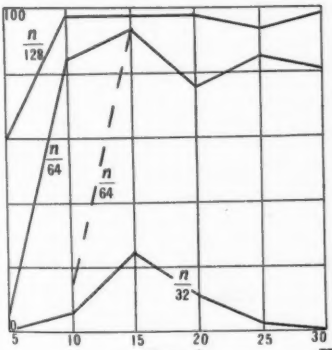


9*b*, Botrytis.

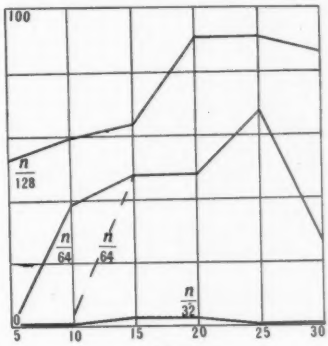


10*b*, Monilia.

H_2SO_4.



9*c*, Botrytis.



10*c*, Monilia.

HNO_3.

at 15° or 20°. This variation may be the result of using solutions that were, without regard to temperature, more injurious to some of the fungi than to others; but strong concentrations, such as $n/4$ and $n/8$ HNO_3, when used with Mucor and Sterigmatocystis, have not given the rise in the curves from 10° to 5° that has been repeatedly obtained with Botrytis and Monilia. It should be noted that these last two fungi are not only the ones that are most greatly injured by the toxic agents, but also are those that require the least stimulus for germination. DUGGAR (**10**) found that both Botrytis and Monilia germinated readily in distilled water, but that Sterigmatocystis and Penicillium did not germinate.

There is also a remarkable agreement in the minimum temperature for the germination of a particular fungus under certain conditions and the location of the fall in its germination curve. Botrytis and Monilia not only show the greatest increase in toxic effect on passing from 5° to 10°, but they are also the only ones that had germinated in the control cultures at 10° by the end of one day. With Penicillium the $CuSO_4$ and HNO_3 curves show a tendency to drop between 10° and 15°, while in the H_2SO_4 curves the fall comes beyond 15°. Along with these data should be noted the fact that the control with the H_2SO_4 cultures gave no germination in one day, while those with the HNO_3 and $CuSO_4$ cultures had germinated in this time. This variation in the controls was probably due to a slight change in the temperature of the refrigerator, together with the fact that 15° approaches the lower limit of temperature for obtaining the germination of Penicillium in one day (WIESNER **13**). Mucor gave no germination the first day in the controls at 15°, with the exception of about 14 per cent. with the $CuSO_4$ series, and Sterigmatocystis in no instance germinated in one day at this temperature. As has been already mentioned, the curves for these fungi fall between 15° and 20° or at a higher temperature. From these facts it is seen that the spores exposed to a harmful agent and at the same time inhibited by cold have not been greatly injured.

In the cultures from which the data in charts *6a* to *10c* inclusive were obtained, the spores were in no instance transferred. The cells in the $CuSO_4$ series remained at the temperature indicated for four days; those with the acids were removed at the end of two days.

In the former six daily observations were made, in the latter only four. Control cultures of spores in beet decoction were kept with the toxic cultures at all times. These controls were subjected to the various temperatures for the same length of time as the other cultures. The per cent. indicated in these charts do not in every instance represent the actual germination, but were in all cases obtained by dividing the per cent. of germination in the particular culture by that in the control at the same temperature. It was found more difficult to represent in graphical manner the results obtained from these experiments, since the per cent. of germination did not always seem to agree with the extent of the injury. The solid lines show the germination at the time of the final removal from the given temperature. The per cent. of germination at the end of twenty-four hours is indicated by the broken lines. These unite with the solid lines as they approach the optimum temperatures. Where no broken line is given the germination was the same at the end of the first day as at the time of removal from the special temperature. The results obtained at temperatures at which the controls had not germinated were omitted from the curves. This accounts for the fact that a number of the curves are not extended to the lower temperatures.

In all instances the injurious effects were least at the optimum for the fungus. This optimum was determined by the germination and development in the controls. The harmful effects were shown by decreased germination as indicated in the charts and by abnormal development. The toxic solutions that gave but partial germination at the optimum for the fungus usually gave only abnormal development above and below that optimum. Thus, Botrytis in $n/32$ HNO_3 gave mycelial development approaching the normal only at 15° and 20°; Penicillium in $n/128$ $CuSO_4$ gave medium growth at 20° but at no other temperature, at 30° the germ tubes seldom became more than a few spore diameters in length even after removal to a more favorable temperature and many spores swelled without germinating. Sterigmatocystis has its optimum above 25° and it is the only fungus in which the injurious effects decreased above that temperature. Both Mucor and Sterigmatocystis germinated and grew well at 35°, the other three fungi gave little or no germination at that temperature.

The charts do not show the results obtained at low temperatures, but in every instance cultures were placed at 5° and 10°. Spores kept for two days in a particular toxic solution at a temperature so cold that it inhibited their germination gave, upon removal to the room temperature, a germination and development that was but little inferior to that obtained from the fresh spores under like conditions of medium and temperature. Spores inhibited at a temperature that did not prevent germination were more greatly injured. Spores of Mucor gave fair growth in $n/32$ H_2SO_4 and $n/32$ HNO_3 after removal from 5°, but after removal from 10° did not germinate. Sterigmatocystis spores in $n/16$ HNO_3 grew almost as well after removal from 10° as from 5°, but in the cultures removed from 15° (a temperature not inhibiting germination in the control) no germination was obtained.

A comparison of the curves obtained with the different chemicals shows that those for weak concentrations of HNO_3 do not drop so rapidly at high temperatures as the curves for weak solutions of the other toxic agents.

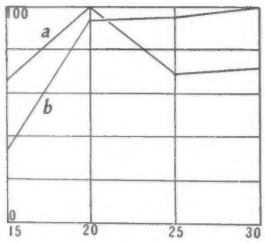
In order to obtain additional information in regard to the significance of the results secured with the cells, a series of flask cultures was made. In every instance 25^{cc} of the given solution were placed in a 100^{cc} flask. These flasks were sterilized after the introduction of the solution. The effect of the toxic agent was determined by taking the dry weight of the fungous growth at the end of the given time. With the exception of $n/64$ $CuSO_4$, duplicate cultures were made and the average weight used in estimating the effect. Flask cultures were made of Mucor, Penicillium, and Sterigmatocystis. The results obtained from cell cultures were used as a basis in determining what strengths of the toxic agents should be used in the flasks. So much greater concentration was required to give injury in flask cultures than in cells that no definite results were obtained with Mucor and Penicillium. Also it was found that Penicillium would not grow in flask cultures placed at 30° C., a temperature at which the fungus grew well in the control cell cultures.

In the series with Sterigmatocystis the air in the incubator was dry, while that in the refrigerator was kept damp by the melting ice. It was feared that the evaporation from the flasks at 25° and 30°

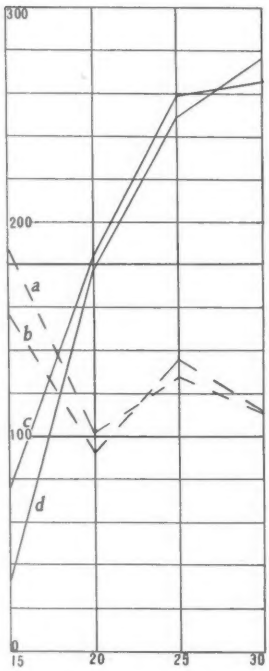
might have been for this reason enough greater than at lower temperatures to cause an appreciable change in the results, and the series was repeated with the hygroscopic conditions more nearly uniform. Charts 12*a* and 11 give the results obtained from the first series, while 11 and 12*b* show the results of the last series. In the first set of experiments the fungus was allowed to grow two weeks, in the last but one week; in the former the cultures for the various temperatures were started at different times, in the latter all were started on the same day and the spores used were from one stock culture.

In charts 11–12*b* the abscissae indicate temperatures as before, but the ordinates express percentages of dry weight instead of percentages of germination. The effect of temperature on the controls is shown in chart 11. In this chart the curves were determined by taking the greatest dry weight as 100 and estimating what per cent. of this the weights secured at other temperatures were. In charts 12*a* and 12*b* these same controls are represented by the ordinates marked 100. In plotting the curves of these two charts, the weight of fungus secured in a given toxic solution at a particular temperature was compared in each case with the weight obtained in the control at the same temperature. The results thus obtained are expressed by the ordinates as mentioned above.

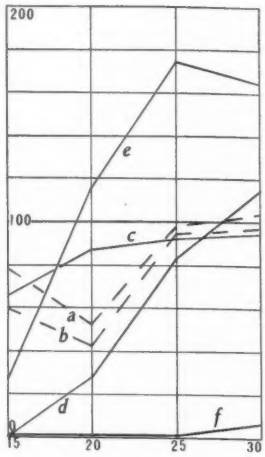
It will be seen that in most instances the curves in the two charts are in close agreement. Where this is not true, as in the case of the controls, the results obtained in the last series should be considered the more reliable for the reason previously given. Taking total growth as a standard, the injurious effects of the toxic agent have decreased with rise of temperature. This decrease is rather to be considered as the result of approaching the optimum for the fungus than as a mere temperature effect. The effects produced by the three chemicals were widely different. The injury resulting in the $CuSO_4$ solutions was not so great, comparatively, at 15° as at 20°. This was true of neither of the acids. Sulfuric acid checked the growth at the lower temperatures, but in no case served as a strong stimulating agent. Nitric acid gave similar injurious effects, but at the higher temperatures served as a remarkable stimulus. It should be remembered that a similar rise at 25° and 30° was obtained in the cell culture curves for HNO_3.



11, Sterigmatocystis. Control curves; *a*, for first series; *b*, for second series.



12*a*, Sterigmatocystis, first series. *a*, *n*/256 $CuSO_4$; *b*, *n*/192 $CuSO_4$; *c*, *n*/32 HNO_3; *d*, *n*/24 HNO_3.



12*b*, Sterigmatocystis, second series. *a*, *n*/128 $CuSO_4$; *b*, *n*/64 $CuSO_4$; *c*, *n*/20 H_2SO_4; *d*, *n*/10 H_2SO_4; *e*, *n*/20 HNO_3; *f*, *n*/10 HNO_3.

The writer hopes by means of further experiments to be able to obtain additional information in regard to the meaning of the difference in action of the chemicals and the significance of the varying effects upon the different fungi. He wishes to acknowledge his indebtedness to Dr. B. M. DUGGAR, Professor of Botany in the University of Missouri, for suggesting the problem reported upon in this paper and for valuable advice throughout the prosecution of the work.

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LITERATURE CITED.

1. THIELE, Die Temperaturgrenzen d. Schimmelpilze 10, 36. 1896.
2. NÄGELI, Die niederen Pilze 30, 200. 1877.
3. HEIDER, Ueber die Wirksamkeit von Desinfektionsmitteln bei höherer Temperatur. Cent. Bakt. **9**: 221–228. 1891.
4. PASTEUR, Compt. Rend. **84**: 206. 1877.
5. COHN, Beitr. z. Biol. Pfl. **2**: 255, 259. 1877.
6. BREFELD, Unters. ü. d. Spaltpilze 10. 1878.
7. RICHET, C., La chaleur animale. Paris. 1889.
8. MATHEWS, A. P., The relation between solution tension, atomic volume, and the physiological reaction of the elements. Amer. Jour. Physiol. **10**: 290–323. 1904.
9. CLARK, J. F., On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. BOT. GAZETTE **28**: 289, 378. 1899.
10. DUGGAR, B. M., Physiological studies with reference to the germination of certain fungous spores. BOT. GAZETTE **31**: 38. 1901.
11. FERGUSON, MARGARET C., A preliminary study of the germination of the spores of *Agaricus campestris* and other basidiomycetous fungi. U. S. Dept. Agric., Bureau Pl. Ind., Bull. 16. 1902.
12. CLARK, J. F., On the toxic properties of some copper compounds with special reference to Bordeaux mixtures. BOT. GAZETTE **33**: 26. 1902.
13. WIESNER, J., Untersuchungen über den Einfluss der Temperatur auf die Entwicklung des *Penicillium glaucum*. Sb. Wien. Akad. **68**: 5–16. 1873.

THE EMBRYOGENY OF SOME CUBAN NYMPHAEACEAE.

MELVILLE THURSTON COOK.

(WITH PLATES XVI–XVIII.)

THE taxonomic position of the Nymphaeaceae has always been somewhat doubtful. The anatomy of these plants is more nearly that of the monocotyledons, while the venation of the leaves would indicate that they are dicotyledons. A few years ago researches in this family were stimulated by LYON'S studies (**17**, **18**) on the embryogeny of Nelumbo, in which he came to the conclusion that they should be classified among the monocotyledonous families in the series Helobiae. LYON'S views were strengthened by my own paper on the embryogeny of *Castalia odorata* and *Nymphaea advena* (**7**), by SCHAFFNER'S paper on morphological peculiarities of the Nymphaeaceae and Helobiae (**26**), and by YORK'S embryological studies on Nelumbo (**28**). But CONARD (**5**, **6**) in his studies in this same family took exceptions to this view, and holds to the idea that they should be classified among the dicotyledons. More recently, MOTTIER (**20**) has declared his belief that they are anomalous dicotyledons. However, he does not claim to have made any study of the Nymphaeaceae, but confines his studies to well recognized dicotyledonous species.

Immediately following LYON'S paper (**18**) on the embryogeny of Nelumbo, CAMPBELL (**4**) raised the question as to whether other genera of the Nymphaeaceae might not also be monocotyledonous, and called attention to the fact that the structure of the flowers and character and arrangement of the vascular bundles in Cabomba and Brasenia were very similar to some of the Alismales; also "that the form of the leaves is often very suggestive of the sagittate leaves of Alisma or Sagittaria."

In consideration of the differences of opinion indicated above the writer accepted an opportunity to make a study of certain tropical species, hoping that additional light might be thrown on this very interesting family.

SCHAFFNER (**26**) called attention to the fact that it is in reality much easier to read monocotyledonous than dicotyledonous charac-

ters into the flowers; that while the genus Castalia has been described as having four sepals, *C. odorata* usually has only three, though sometimes by an expansion of the receptacle one segment of the second cycle is more or less exposed, while in *C. tuberosa* the displacement is normal. This led me to make a similar study of *C. pubescens*. In this species I found that 80 per cent. have the first segment of the second cycle exposed, while 20 per cent. have the second segment of the second cycle more or less exposed, thus showing five parts. SCHAFFNER also called attention to certain other secondary resemblances, such as number and arrangement of ovules and ovularies, between the Nymphaeaceae and well-recognized monocotyledonous plants.

The vascular bundles of all the species referred to in this paper were also studied, but gave no facts other than those already well-known, the bundles in all cases being of the well-recognized closed type and arranged in the stems after the usual manner of this family.

On account of the large amount of gummy substance surrounding the ovules, considerable difficulty was experienced in getting a killing fluid to penetrate. This was especially true for *Brasenia purpurea* where the gummy substance was most abundant. Picric-acetic solutions proved to be the best fixing agents, while chrom-acetic and the Flemming's solutions were unsatisfactory and could be used only for the development of the embryo sac and the stamens.

THE EMBRYO SAC.

The formation of the embryo sacs in all genera is very similar, and in fact practically the same as described in my first paper (7). I will not give a discussion of each, therefore, but will give a general outline and present a series of figures illustrating the more important points, with special attention to the mature sac and the changes at the time of fertilization and immediately following.

The archesporial cell develops from the hypodermis (*fig. 11*) and is easily recognized. An indefinite number of tapetal cells, usually varying from four to eight, are then produced. Three or four megaspores are then formed. The four may be produced either by a regular division of the mother cell into four cells; or occasionally by the division of the mother cell into three cells, followed by a division of the middle one (*fig. 2*).

The innermost megaspore is functional and develops rapidly into the embryo sac at the expense of the other megaspores and the tapetal cells. This enlarged cell (*fig. 3*) now touches the epidermis at the micropyle and passes rapidly through the two-, four-, and eight-nucleate stages. The mature embryo sac is very small and straight, and the enlargement is principally in the direction of the long axis of the ovule. Starch is usually very abundant and persists throughout the two- and four-nucleate stages, and in *Brasenia purpurea* and *Cabomba piauhiensis* usually throughout the eight-nucleate stage. It gradually disappears, first from the micropylar end of the sac and finally from the antipodal end (*fig. 6*). It probably undergoes modification to form the first food for the development of the embryo and endosperm.

Occasionally two embryo sacs were produced, but one was always absorbed by the other (*fig. 7*). This was more frequent in *Cabomba piauhiensis* than in any other species studied. Fertilization occurs almost immediately upon the completion of the eight-nucleate stage of the sac. The same very pronounced sclerification of the inner part of the epidermal cells (*fig. 4*), previously observed by me (**7**) for *Castalia odorata*, was observed in the tropical species of Castalia studied, but not in the other genera. The actual penetration of this epidermal wall by the pollen tube was observed in only a few cases. In fertilization the pollen tube (*fig. 4*) enlarged and stained so deeply that it was impossible to observe what fusion of nuclei did occur. At the time of fertilization the polar nuclei are very large, usually rather indistinct, and unite at the micropylar end of the sac just below the egg apparatus. At the same time the antipodals, which are very inconspicuous, undergo degeneration (*fig. 4*). The synergids may persist a short time after fertilization (*figs. 5, 6, 7*), but usually disappear very quickly. They are most persistent in *Cabomba piauhiensis*.

The primary endosperm nucleus, which is now very large and conspicuous, moves to the antidopal end of the sac (*figs. 5, 6*), where it divides (*figs. 7, 8, 20*), and a very delicate wall is formed across the sac between the two daughter nuclei. This wall can be observed without great difficulty in *Nymphaea advena* (*fig. 8*) and in *Castalia ampla* (*fig. 20*), but is very difficult to see in the other species studied.

The nucleus next to the embryo divides repeatedly, thus forming the endosperm which will be described later. The nucleus in the antipodal end acts slightly differently in the different species and will hereafter be designated the nucleus of the nucellar tube.

In *Nymphaea advena* a long tube-like extension of the sac is now formed through the nucellus, beginning with the antipodal end of the sac and extending to the chalazal end of the ovule. The very large and conspicuous daughter nucleus of the antipodal end, formed by the division of the endosperm nucleus, enters this tube, which will be called the nucellar tube, and travels to the chalazal end of the ovule, where it undergoes disintegration (*figs. 8, 12, 13, 14*). When the embryo is in the two-celled stage, the tube and nucleus have traveled about two-thirds the length of the ovule (*figs. 8, 12*); and when the embryo has reached the quadrant or octant stage, the tube is complete (*figs. 13a, 13b*). In one case only (*fig. 13b*) the tube nucleus had divided. After this time it disintegrates (*fig. 14*). In its early development the tube is filled with protoplasm which to all appearances is traveling towards the embryo sac. This is exactly the condition observed by me in *Nymphaea advena* and *Castalia odorata* and described in my first paper (**7**).

In *Castalia ampla* (*fig. 20*) the nucellar tube is a short thick sac which is separated from the embryo sac by a constriction and by a thin wall, and which contains a very large nucleus and a large amount of protoplasm. As the embryo increases in size, the embryo sac enlarges, encroaching upon this nucellar tube sac and absorbing its contents (*fig. 21*). The formation of this tube sac is very similar to that described by JOHNSON (**12**) for *Saururus cernuus*, except that in that species the tube sac is relatively larger and persists in the mature seed.

In *Castalia pubescens* the nucellar tube is much slower in development, much less conspicuous, and apparently of much less importance than in any of the other species studied. Extending from the antipodal end of the sac to the chalazal end of the ovule is a great mass of elongated cells (*fig. 24*) which are much richer in protoplasmic contents than the other cells of the nucleus. The greater part of this mass of cells disintegrates slowly and thus is formed a small tube reaching usually not more than one-half the length of the ovule

(*fig. 9a*). The tube is flat (*fig. 9b*) instead of cylindrical as in the case of the other species. This tube is usually completed just before the appearance of the cotyledonary ridge of the embryo. The nucleus was observed in the antipodal end of this tube but apparently disintegrated, and did not pass to the opposite end of the tube as in the case of the other species.

In *Brasenia purpurea* and *Cabomba piauhiensis*, the nucellar tube is long but very small. There is a rapid disintegration of the nucellar tissue to form the tube before the tube nucleus begins its passage. The tube nucleus gradually disintegrates, and has entirely disappeared before it reaches the chalazal end of the tube (*figs. 7, 10, 11*); the chalazal end of the tube is considerably enlarged, is not so definite in outline as in *Nymphaea advena*, and contains fragments of the cell walls (*fig. 11*).

In my previous discussion (**7**) I called attention to the fact that the behavior of the endosperm nucleus is similar to that of *Sagittaria variabilis* as described by SCHAFFNER (**25**), except that in *S. variabilis* there is no nucellar tube elongation; also, that judging from the studies of WIEGAND (**27**) and HOLFERTY (**10**) on Potamogeton it is possible that a similar condition may be found in that genus.

Since that time, HALL (**9**), in his studies on *Limnocharis emarginata*, in which he finds a single polar nucleus, says:

> The upper polar nucleus, when it has approached the antipodal end of the sac, divides transversely. The lower daughter nucleus remains in the position of its formation, being cut off by a wall across the sac and forming a large cell which does not divide further, but finally disappears through the encroachment of the endosperm. . . . . The upper daughter nucleus travels back towards the egg apparatus, and by its further division forms the endosperm.

STRASBURGER (**23**) describes a similar action of the endosperm nucleus of *Ceratophyllum submersum*, in which, after the first division, the nucleus in the antipodal end of the sac does not divide, while the nucleus of the micropylar end forms the endosperm.

CAMPBELL (**1**) in his discussion of *Naias flexilis* and *Zannichellia palustris* describes a condition which further study may prove to be similar to the Nymphaeaceae. In discussing Naias he says:

> A peculiarity noted, which was also observed in Zannichellia, was the presence of a single large nucleus close to the antipodals, which was conspicuous at an early period and behaved much like the nucleus of the suspensor. Whether this

was the lower polar nucleus or one of the two endosperm nuclei resulting from the first division of the primary endosperm nucleus could not be determined. Whichever is the case, it never divides, and all the endosperm nuclei arise from the division of the other primary endosperm (or polar) nucleus. The endosperm is limited, there being usually no trace of cell formation.

In discussing *Zannichellia palustris* he says:

As in Naias, there is evident soon after fertilization a large nucleus just above the antipodal cells, which undergoes no division, but increases very much in size. This is more variable in size than in Naias; not infrequently it could not be detected in the later stages, and in several instances it looked as if it were undergoing disintegration.

Previous to this time, JOHNSON (**12**) had published his studies on *Saururus cernuus*, in which he described a division and behavior of the endosperm nucleus very similar to what I described for Nymphaeaceae, and the formation of a nucellar tube sac intermediate between what I have described in this paper for *Nymphaea advena*(?) and *Castalia ampla*. JOHNSON (**15**) has since called attention to other genera of the Saururaceae (Anemiopsis and Houttuynia) which possess this character.

In my previous discussion of this subject (**7**), I expressed the opinion that the physiological significance of this nucellar tube and nucleus presented a very interesting problem, which should be considered in connection with the function of the antipodals. I called attention to the fact that in Ranunculaceae, Sparganium, and Vaillantia the antipodals appeared to furnish nourishment for the embryo; that the peculiar haustorial development of the antipodals of Vaillantia, the enlargement of the lower antipodal in Aster, the accumulation of endosperm in the antipodal region of Alyssum, and the large lower nucleus formed by a division of the endosperm nucleus (nucellar tube nucleus) in Sagittaria and the Nymphaeaceae showed a resemblance which I believed to indicate a similar physiological function. At about the same time IKEDA (**11**) published the results of his studies on the physiological functions of the antipodals, in which he demonstrated by microchemical observations that the antipodals of Liliaceae possessed very important physiological functions.

JOHNSON (**14**) also considers the antipodals of considerable physiological importance in certain of the Piperaceae, in which he describes them as increasing in size and sometimes in number. It

seems very evident that this nucellar tube and its nucleus, which has common origin with the endosperm nuclei, are important structures for supplying food to the embryo through the agency of the endosperm. In *Castalia pubescens*, where the tube is developed, the same function is performed, first by the axial core of elongated cells (*fig. 24*) and later by the small nucellar tube (*fig. 9*).

YORK (**28**) in his studies on Nelumbo did not observe the formation of a nucellar tube with its nucleus, but claims that both nuclei formed by the first division of the primary endosperm nucleus divided repeatedly. However, it may be that YORK failed to observe the first division of this endosperm nucleus, that the tube nucleus disintegrates very quickly as in *Castalia pubescens*, and that he really observed the secondary divisions of the daughter endosperm nucleus in the micropylar end of the sac.

ENDOSPERM.

The development of the endosperm in this family presents two distinct types, Nymphaea, Castalia, and Nelumbo illustrating one, and Brasenia and Cabomba the other. The first type shows the formation of cell walls, that is, a cellular endosperm throughout its entire development; while the second type forms cell walls only in the latter part of its development. The formation of the two types of endosperm by closely related plants has been noted by several investigators.

In the first type the first cell walls are formed across the sac (*figs. 13a, 20, 23*), but gradually become more irregular and extend in various planes (*figs. 21, 24, 25*). At first the cells contain considerable protoplasm and the entire endosperm is very active, but in a short time it seems to have reached its maximum activity and importance. In *Castalia pubescens*, at about the time and following the appearance of the cotyledonary ridge, the endosperm cells immediately over the plumule show a marked difference from the surrounding cells (*fig. 9a, x*). They are slightly smaller, more delicate, and are probably the cells which give nourishment directly to the embryo. In this same species the endosperm may penetrate the antipodal end of the nucellar tube a short distance (*fig. 23*).

In the second type, represented by Brasenia and Cabomba, the

protoplasm is very dense and the cells at first divide rapidly, but no cell walls are formed (*figs. 10, 33b*). After the embryo has passed the stage indicated in *fig. 34*, the endosperm appears to become thinner and does not stain readily until the embryo is near maturity. The protoplasm then becomes very dense, cell walls are formed, and one layer (occasionally two or three in Brasenia) of cells is developed which completely surrounds the embryo (*fig. 40*). This endosperm is usually thicker and forms two or more layers in a zone around the embryo at the point of origin of the cotyledonary lobes (*fig. 38, x*), and are very thin just below the root tip (*fig. 38, y*). It appears that the endosperm in Brasenia and Cabomba must perform a more important function in the germination of the embryo than in the species of the other genera.

JOHNSON (**14**) in his studies on Piperaceae has expressed the opinion that the "embryo sporophyte of the second generation is never nourished by the parent sporophyte directly, but always through the intermediate gametophyte." The development and action of the endosperm in Nymphaeaceae confirms JOHNSON'S conclusions.

In all species studied there is a pronounced lateral enlargement of the embryo sac, at the expense of the nucellus, to accommodate the increasing endosperm and growing embryo (compare *figs. 24* and *25*).

EMBRYO.

The development of the embryo shows a very wide range of variation. The embryo of *Nymphaea advena* (?) of Cuba follows almost exactly the same course as previously described by the writer for *Nymphaea advena* of the northern United States. The fertilized egg first divides transversely (*fig. 12*), and then two longitudinal walls result in a spherical embryo of eight cells. Successive cell divisions occur, but the spherical character is retained for some time, after which there is an excessive growth on the side next to the micropyle, forming a suspensor by which the embryo is attached to the nucellus, and a flattening on the opposite side, thus giving the embryo the shape of a short blunt cone, or rather of a pear (*fig. 16*). In this respect it differs from *N. advena* of the north, in which the suspensor is much more rudimentary and does not develop until much later. At this

time it somewhat resembles the embryo of *Sparganium simplex* as described by CAMPBELL (**3**); but without the younger stages of *S. simplex* and more of the young stages of *N. advena* (?) it is impossible to say whether this resemblance is more than superficial. From the large end of this conical embryo a cotyledonary ridge is now produced which extends almost entirely around this end and almost completely encloses the plumule (*fig. 17*), while in *N. advena* of the north it extends only a little more than half the distance around the embryo. This monocotyledonous character is very evident in embryos dissected out of the sacs. Two cotyledonary lobes are next developed from this cotyledonary ridge, thus giving it the dicotyledonous character (*fig. 18*). The development of this dicotyledonous character is much earlier and much more pronounced than in *N. advena* of the north. A number of embryos were cross sectioned and examined very carefully and considerable variation was found in the prominence of this character. In one case it was so great as to give the appearance of two equal cotyledons (*fig. 19*). The development is strikingly similar to that of Nelumbo as described by LYON (**18**) and YORK (**28**).

SCHAFFNER (**26**) dissected the advanced embryos of *N. advena* out of the sacs and clearly demonstrated the formation of the two cotyledonary lobes. He did not contradict my conclusions, as stated by MOTTIER (**20**), but made his studies from older embryos than I was able to secure at the time my studies were made.

The embryos of the two species of Castalia show some differences and also differ from *Castalia odorata*. In *Castalia ampla* a proembryo is formed which may consist of as many as six cells in linear arrangement (*figs. 20, 21*). The terminal cell then divides by a longitudinal wall, which is followed by a similar division in the next cell (*fig. 21*). The four cells thus formed then divide by a second longitudinal wall at right angles to the first. By repeated division, this mass of cells now forms a spherical embryo supported by a suspensor of four or five cells in linear arrangement (*fig. 22*). One or more of these suspensor cells, usually the basal, may divide longitudinally. On account of the seed pods sinking soon after fertilization it was impossible to follow the development in this species further.

In *Castalia pubescens* the embryo develops in the same manner,

except that there are usually not more than four cells in the proembryo. With the formation of the spherical embryo the suspensor increases in diameter and the cells usually divide longitudinally (*figs. 24, 25, 26*). After the spherical stage the embryo gradually assumes a pear-shape, and a little later develops the collar-like ridge which extends about two-thirds around the embryo at its greatest circumference. This condition was readily demonstrated by two series of longitudinal sections cut at right angles to each other and a series of cross sections (*figs. 27–30*). *Fig. 27* is from a longitudinal section passing through the middle (*x*) and between the two points of the crescent-shaped cotyledonary ridge (*y*). *Fig. 28* is from a longitudinal section at right angles to *fig. 27* and passes through the cotyledonary ridge near the points of the crescent (*z*). *Fig. 29* is from a series of cross sections of an embryo of corresponding age to *figs. 27* and *28*. *Fig. 30* is from section *d* of *fig. 29*, i. e. at about the point where the cotyledonary ridge arises. *Fig. 31* is reconstructed from a series of sections of a slightly older embryo. At this time there was no external indication of the two cotyledonary lobes, but the rapid division of cells just within the points of the crescent-shaped cotyledonary ridge (*fig. 30c, 1*) indicates their early formation.

It was impossible to follow the development of the embryo beyond this point, because of the withdrawal of the seed pods from the surface of the water to the bottom, where they were quickly buried in the mud. This withdrawal commenced soon after pollination and was accomplished by the spiral-like formation of the peduncle which gradually contracts. However, SCHAFFNER (**26**) was able to dissect the young embryos of *Castalia odorata* out of their sacs and makes the following statement concerning them:

> Although the "dicotyl" appearance is quite strong, the embryo must be regarded as the same type as Nymphaea and Nelumbo. In the very young embryo there is an expansion which extends nearly around the base but is discontinuous at one side. Soon this expansion develops on opposite sides as two prominent lobes in such a manner that the original connection between the two lobes is very difficult to distinguish. On examining the embryo from below, however, similarity to the Nelumbo and Nymphaea embryos becomes perfectly apparent. There is the same opening on one side, and on the back a connection of the two lobes, only to a less extent. Unless special care were taken in recon-

structing such an embryo from serial sections, one might readily take it for a dicotyl. It will be evident, however, from a comparison of the figures that the Castalia embryo represents only the extreme of the lobing shown in Nelumbo and Nymphaea.

The formation of the suspensor is entirely different from what I observed in *C. odorata*, but corresponds with CONARD'S (**5**, **6**) observations. The suspensor disappears soon after the formation of the cotyledonary ridge.

The development of the embryos of *Brasenia purpurea* and *Cabomba piauhiensis* is practically the same, but since *C. piauhiensis* is much more easily sectioned and furnished much better preparations, most of the drawings were made from it. The fertilized egg divides by the formation of cross walls and produces a proembryo of three or four cells in linear arrangement (*figs. 32, 33a, 34, 35*). The terminal cell then divides, forming a quadrant (*fig. 33a*), then it forms the octant, and then a large spherical embryo supported by a short suspensor of two or three cells which usually divide longitudinally (*figs. 34, 35, 36*). As the embryo increases in size, it becomes more or less flattened against the walls of the sac and develops the cotyledonary ridge (*fig. 36, x*) similar to the two genera just described, except that this ridge extends almost entirely around the plumule, thus forming a pit with the plumule in the center. The dicotyledonous character produced by the development of the cotyledonary lobes appears very early. Only by the most careful examination of the intermediate stages at the time of the first appearance of the cotyledons, and by the most careful cross sections was it possible to demonstrate the common origin of these two cotyledonary lobes. They develop very early and the edges and tips come together, thus enclosing the plumule in a short, hollow cone (*fig. 37*). After this the development of the embryo is a mere increase in size (*figs. 38, 39*), accompanied by the modification of the endosperm previously referred to. The suspensor persists until the embryo is almost mature and then disintegrates.

It will be noted that the young embryo of *Nymphaea advena* (?) is similar to the embryos of *Lysichiton kamtschatense* as described by CAMPBELL (**2**); and of *Ceratophyllum submersum* as described by STRASBURGER (**23**). The young embryos of *Castalia ampla*, *C.*

pubescens, *Brasenia purpurea*, and *Cabomba piauhiensis* all have the same general character as *Nymphaea advena*, except that they possess suspensors. They also show some resemblance (which may be superficial) to the embryo of *Sparganium simplex* as described by CAMPBELL (**3**). They also resemble the embryos of *Naias flexilis* and *Zannichella palustris* as described by CAMPBELL (**1**), *Potamogeton foliosus* and *P. natans* as described by WIEGAND (**27**) and HOLFERTY (**10**), and *Limnocharis emarginata* as described by HALL (**9**) except that they do not possess the large basal cell of the suspensor. The late development of the suspensor in *Nymphaea advena* (?) of Cuba and *N. advena* of the north, as previously described by me, may confirm in some measure COULTER and CHAMBERLAIN'S view that the Alisma-type of monocotyledonous embryo is primitive and that the suspensor in the Pistia type has been suppressed.

It will also be noted that the origin of the cotyledonary lobes in all cases is from a crescent-shaped cotyledonary ridge about the larger part of the embryo, and that my results coincide with the conclusions reached by LYON, SCHAFFNER, and YORK. This point in CONARD'S studies is illustrated by a single figure (*48*, *d*) of *Castalia caerulea*. This figure agrees exactly with *fig. 30* and text *fig. 2*, *d*, *e* of my first paper, with LYON'S *fig. 10*, and YORK'S *fig. 33* of Nelumbo. However, my figures are parts of series which illustrate the true monocotyledonous character of the embryo; and this monocotyledonous character of *C. odorata* was afterwards demonstrated by SCHAFFNER who dissected the embryos out of their sacs.

RICHARD (**21**) referred to the embryos of the grasses, Nelumbo, Ruppia, Hydrocharis, and Zostera as *embryons macropodes*, and SCHAFFNER (**26**) confirms this view and expresses the opinion that the massive expansion and lobes of Halophila, Ruppia, Zostera, Nelumbo, Nymphaea, and Castalia are not homologous with the cotyledons, but are specially developed absorbing organs.

The figures of this paper show a striking resemblance to the figures of anomalous dicotyledons by LEWIS (**16**) and MOTTIER (**20**). However, the flowers show more monocotyledonous than dicotyledonous characters, while only the leaves may be considered as uniformly presenting dicotyledonous characters. It appears, therefore, that the Nymphaeaceae can be more properly classed as

anomalous monocotyledons, rather than as anomalous dicotyledons as suggested by MOTTIER.

SUMMARY.

1. The development of the embryo sac is practically the same in all species.

2. In all species the endosperm nucleus divides and the daughter nucleus in the antipodal end enters a nucellar tube or sac which penetrates the nucellus.

3. This nucellar tube or sac is apparently for the purpose of transferring food from the nucellus to the endosperm, from which it is transferred to the embryo.

4. The endosperm is of two types: the cellular in Nymphaea and Castalia, and the nuclear followed by the cellular in *Brasenia purpurea* and *Cabomba piauhiensis*.

5. The character of the endosperm in the late stages of *Brasenia purpurea* and *Cabomba piauhiensis* indicates a greater importance in germination than in the other genera.

6. The embryo of *Nymphaea advena*(?) originates as a spherical mass of cells and later develops a cotyledonary ridge and suspensor, while the other species develop embryos consisting of a single row of cells, from which is formed a spherical embryo supported by a suspensor. A crescent-shaped cotyledonary ridge is then developed, ranging from two-thirds to almost the entire distance around the embryos.

7. With the development of the cotyledonary ridge, two cotyledonary lobes are produced which may readily be mistaken for two cotyledons.

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NOTE: The material from which these studies were made was submitted to specialists in taxonomy. There was some difference of opinion as to whether the Nymphaea was a large-leaved form of *N. advena* or another closely related species. *Castalia pubescens* is an introduced species from eastern India, Philippines, Java, and Australia. It was collected in a large lagoon near San Antonio de las Baños in Havana Province. *Nymphaea advena*(?) was collected in the river at San Cristobal and in a small lagoon south of Herredura in Pinar del Rio Province; all other species in this same small lagoon. Specimens of all these species have been deposited in the New York Botanical Garden.

LITERATURE CITED.

1. CAMPBELL, D. H., A morphological study of Naias and Zannichellia. Proc. Calif. Acad. Sci. III. **1**:1–62. *pls. 1–5.* 1897.
2. ——— Notes on the structure of the embryo sac in Sparganium and Lysichiton. BOT. GAZETTE **27**:153–166. *pl. 26.* 1899.
3. ——— Studies on the flower and embryo of Sparganium. Proc. Calif. Acad. Sci. III. **1**:293–328. *pls. 46–48.* 1897.
4. ——— On the affinities of certain anomalous dicoyledons. Amer. Nat. **36**:7–12. *figs. 2.* 1902.
5. CONARD, H. S., Note on the embryo of Nymphaea. Science N. S. **15**:316. 1902.
6. ——— The water lilies: a monograph of the genus Nymphaea. The Carnegie Institution of Washington, Publication No. 4. 1905.
7. COOK, MEL. T., Development of the embryo sac and embryos of *Castalia odorata* and *Nymphaea advena*. Bull. Torr. Bot. Club **29**:211–220. *pls. 12–13.* 1902.
8. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. 1903.
9. HALI, J. G., An embryological study of *Limnocharis emarginata*. BOT. GAZETTE **33**:214–219. *pl. 9.* 1902.
10. HOLFERTY, G. M., Ovules and embryo of *Potamogeton natans*. BOT. GAZETTE **31**:339–346. *pls. 2–3.* 1901.
11. IKEDA, T., Studies in the physiological functions of the antipodals and related phenomena of fertilization in Liliaceae. I. *Trycirtis hirta*. Coll. Agric. Imp. Univ. Tokyo **5**:41–72. *pls. 3–6.* 1902.
12. JOHNSON, D. S., On the development of *Saururus cernuus*. Bull. Torr. Bot. Club **27**:365–372. *pl. 23.* 1900.
13. ——— On the endosperm and embryo of *Peperomia pellucida*. BOT. GAZETTE **30**:1–11. *pl. 1.* 1900.
14. ——— On the development of certain Piperaceae. BOT. GAZETTE **34**: 321–340. *pls. 9–10.* 1902.
15. ——— Seed development in the Piperaceae and its bearing on the order. Johns Hopkins Univ. Circ. **178**:29–32. 1905.
16. LEWIS, C. E., Studies on some anomalous dicotyledonous plants. BOT. GAZETTE **37**:127–138. *pls. 7–8.* 1904.
17. LYON, H. L., Preliminary note on the embryogeny of Nelumbo. Science N. S. **13**:470. 1901.
18 ——— Observations on the embryogeny of Nelumbo. Minn. Bot. Studies **2**:643–655. *pls. 48–50.* 1901.
19. ——— The embryo of the angiosperms. Amer. Nat. **39**:13–34. *figs. 11.* 1905.
20. MOTTIER, D. M., The embryology of some anomalous dicotyledons. Annals of Botany **19**:447–463. *pls. 26–27.* 1905.

21. RICHARD, L. C., Analyse botanique des embryons endorhizes ou monocotyledonés, et particulièrement de celui des Graminées. Annales Paris Mus. Nat. Hist. **17**:223–251, 442–487. 1811.

22. SARGENT, ETHEL, Evolution of the monocotyledons. BOT. GAZETTE **37**: 325–345. *figs. 6.* 1904.

23. STRASBURGER, E., Ein Beitrag zur Kenntniss von *Ceratophyllum submersum* und philogenetische Erörterungen. Jahrb. Wiss. Bot. **37**:477–526. *pls. 9–11.* 1902.

24. SCHAFFNER, J. H., The embryo sac of *Alisma Plantago*. BOT. GAZETTE **21**:123–132. *pls. 9–10.* 1896.

25. ——— The life history of *Sagittaria variabilis*. BOT. GAZETTE **23**:252–273. *pls. 20–26.* 1897.

26. ——— Some morphological peculiarities of the Nymphaeaceae and Helobiae. Ohio Nat. **4**:83–92. *pls. 5–7.* 1904.

27. WIEGAND, K. M., The development of the embryo sac in some monocotyledonous plants. BOT. GAZETTE **30**:25–47. *pls. 6–7.* 1900.

28. YORK, H. H., The embryo sac and embryo of Nelumbo. Ohio Nat. **4**:167–176. 1904.

EXPLANATION OF PLATES XVI–XVIII.

Figures of the same relative value are drawn with the same lenses: *figs. 1–7* with Zeiss no. 4 oc. and $\frac{1}{12}$ obj.; *figs. 12–16, 20–28, 30, 32, 36,* and *40* with no. 4 oc. and DD obj.; *figs. 8–11, 17–19, 29, 31, 37–39* with no. 4 oc. and A obj.

PLATE XVI

FIG. 1. Archesporial cell of *Brasenia purpurea.*

FIG. 2. Megaspores of *Nymphaea advena* (?).

FIG. 2. Uninucleate embryo sac of *N. advena* (?).

FIG. 4. Mature embryo sac of *Castalia pubescens*, showing entrance of pollen tube (*pt*).

FIG. 5. Embryo sac of *C. pubescens* just after fertilization, showing fertilized egg (*o*), remains of synergids (*syn*), and the endosperm nucleus.

FIG. 6. Embryo sac of *Brasenia purpurea* just after fertilization, showing egg (*o*), synergid (*syn*), endosperm nucleus (*end nu*), and starch in the antipodal end of the sac.

FIG. 7. Embryo sac of *Cabomba piauhiensis*, showing fertilized egg (*o*), the two synergids (*syn*), the endosperm nucleus (*end nu*) after first division, and the remains of a second sac (*e s*).

FIG. 8. Embryo sac of *N. advena* (?), showing two-celled embryo, the nucellar tube, and the two daughter cells of the endosperm nucleus (*en*) separated by a wall; the tube is about three-fourths the length of the ovule.

FIG. 9*a*. Embryo sac of *C. pubescens*, showing large pear-shaped embryo with region of modified endosperm (*x*), and the nucellar tube.

FIG. 9*b*. Cross-section of nucellar tube of 9*a*.

FIG. 10. Embryo sac of *C. piauhiensis*, showing two-celled embryo and nucellar tube. The endosperm nucleus has divided and one daughter cell entered the tube (*ntn*), while the other has remained in the sac and undergone first division (*end*).

FIG. 11*a*. Embryo sac and mature nucellar tube of *C. piauhiensis* with spherical embryo; the thin endosperm filled the sac at this time.

FIG. 11*b*. Cross-section of tube of *11a* at smallest part.

FIGS. 12–19. *Nymphaea advena*(?).

FIG. 12*a*. Two-celled embryo with daughter nucleus of endosperm nucleus (*en*) after first division; same as *fig. 8*.

FIG. 12*b*. Nucellar tube nucleus of *figs. 8* and *12a*.

PLATE XVII

FIG. 13*a*. Section of spherical embryo in octant stage surrounded by endosperm.

FIG. 13*b*. Lower part of the nucellar tube of *13a* in which the nucellar tube nucleus was divided.

FIG. 14. Mature nucellar tube nucleus.

FIG. 15. Spherical embryo.

FIG. 16. Pear-shaped embryo at time of origin of cotyledonary ridge and with well-developed suspensor.

FIG. 17. Cross-section of embryo a little older than that in *fig 16*.

FIG. 18. Cross-section of embryo older than that in *fig. 17*, showing monocot character in lower part and dicot character in upper part; 3d, 4th, 6th, 14th, 19th, and 24th sections.

FIG. 19. Cross-section of embryo showing equal cotyledonary lobes (dicot characters); 1st, 4th, 5th, 7th, 8th, and 15th sections.

FIGS. 20–22. *Castalia ampla*.

FIG. 20. Embryo sac and two-celled embryo showing endosperm and nucellar tube nucleus (*ntn*) in sac-like nucellar tube.

FIG. 21. Older stage of same, showing absorption of nucellar tube and nucleus (*ntn*) by endosperm.

FIG. 22. Spherical embryo with suspensor.

PLATE XVIII

FIGS. 23–31. *Castalia pubescens*.

FIG. 23. Two-celled embryo and endosperm; the endosperm has penetrated the upper part of the nucellar tube.

FIG. 24. Spherical embryo with suspensor and endosperm, also showing elongated nucellus cells in axis of ovule below sac (*enc*).

FIG. 25. Older stages of the same showing enlargement of sac at right angles to the original long axis.

FIG. 26. Young pear-shaped embryo.

FIG. 27. Longitudinal section of embryo passing through middle of crescent-shaped cotyledonary ridge (*x*) and between the points of the same (*y*).

FIG. 28. Longitudinal section at right angles to *fig. 27* passing through the cotyledonary ridge (*zz*) near the points of the crescent.

FIG. 29. Cross sections of embryo at about same age as *figs. 27* and *28*.

FIG. 30. Section *d* of *fig. 29; cl*, cotyledonary lobes; other letters same as in *figs. 27* and *28*.

FIG. 31. Reconstruction from sections of embryo a little older than those in *figs. 27–30*.

FIGS. 32–40. *Cabomba piauhiensis*.

FIG. 32. Two-celled embryo.

FIG. 33*a*. Section of embryo in octant stage showing two-celled suspensor.

FIG. 33*b*. Endosperm of 33*a*.

FIG. 34. Spherical embryo with three-celled suspensor.

FIG. 35. Spherical embryo showing original two-celled suspensor subdivided by longitudinal divisions.

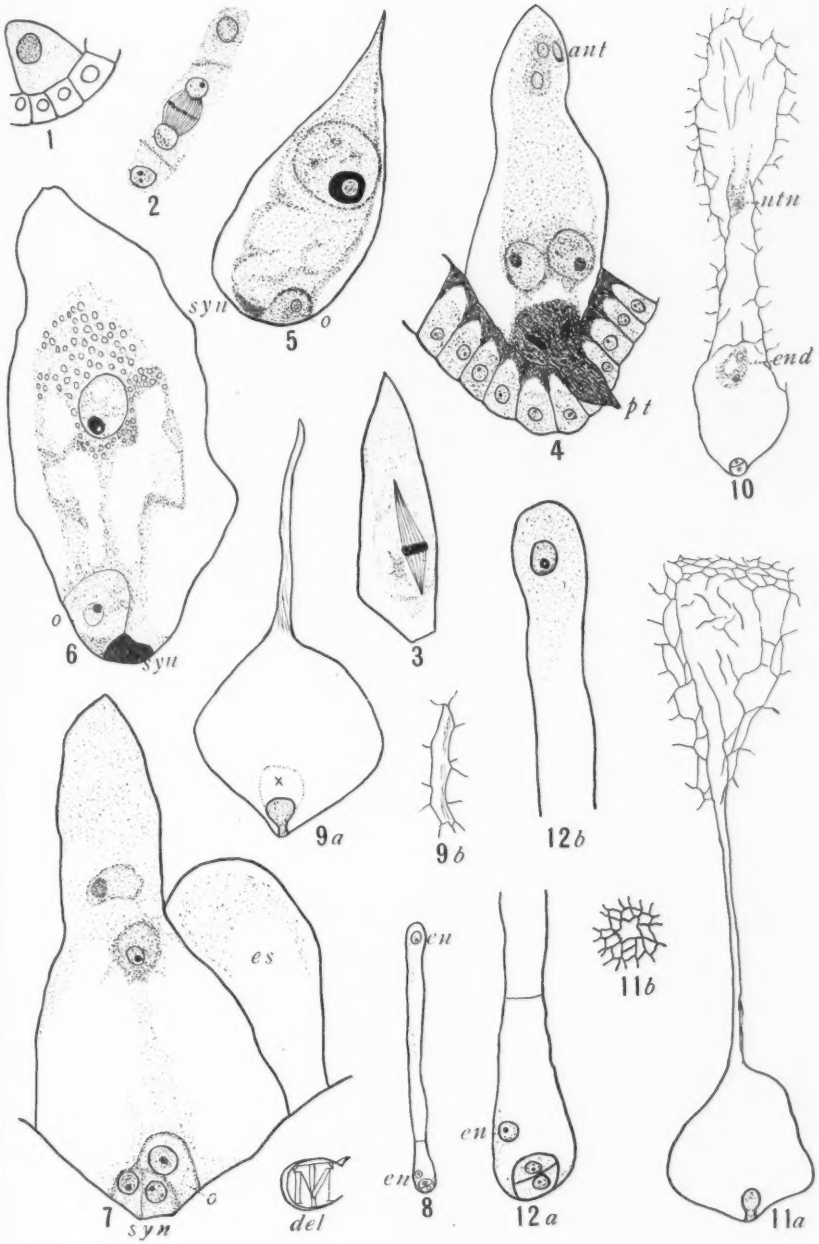
FIG. 36. Longitudinal section of embryo at about time of origin of cotyledonary ridge, showing two-celled suspensor; cut same direction and showing same points as *fig. 27*.

FIG. 37. Reconstruction from section of embryo slightly older than *fig. 36* and showing early development of cotyledonary lobes.

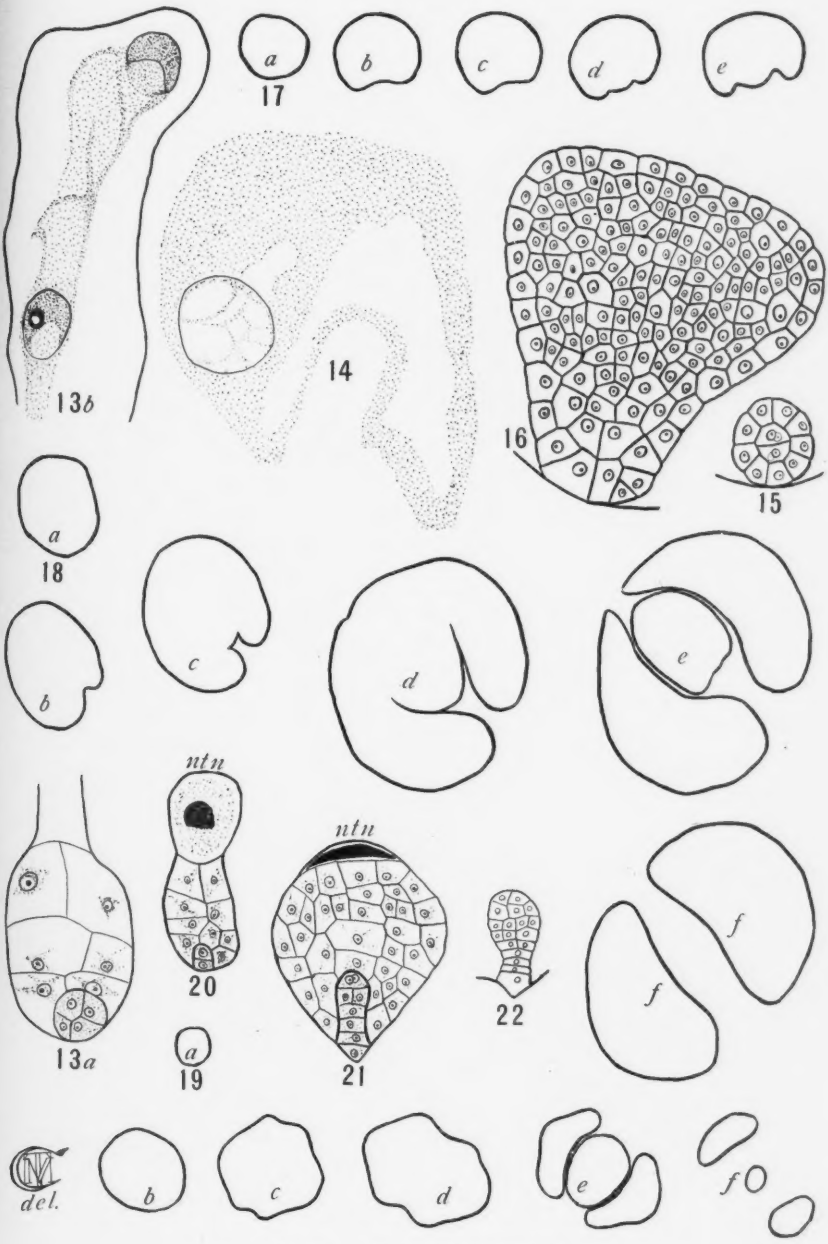
FIG. 38. Reconstruction from sections of almost mature embryo; drawn to same scale as *fig. 37*.

FIG. 39. Cross-sections of embryo; 2d, 6th, 8th, 12th, 24th, and 28th sections.

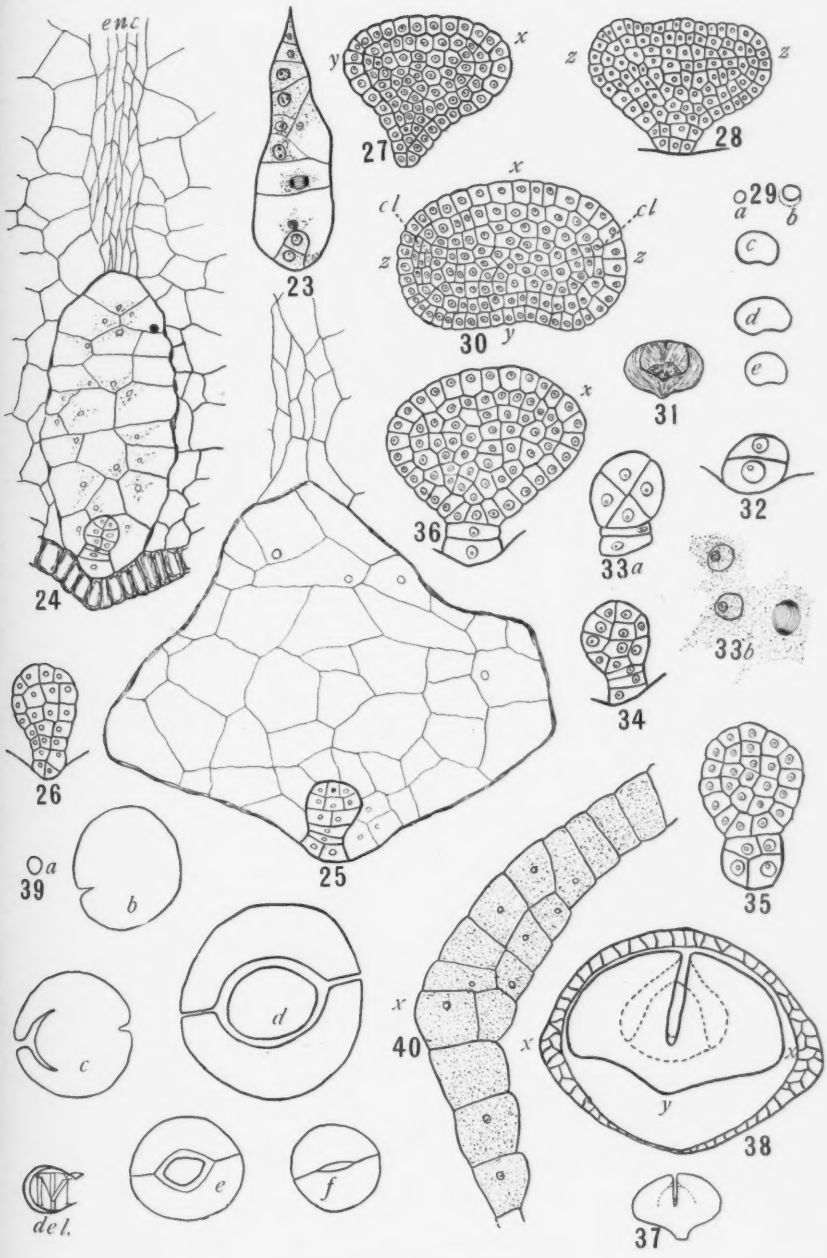
FIG. 40. Endosperm from embryo near same age or little older than *fig. 38*.



COOK on NYMPHAEACEAE



COOK on NYMPHAEACEAE



COOK on NYMPHAEACEAE

CURRENT LITERATURE.

BOOK REVIEWS.

Two new western "Floras."

DR. RYDBERG has long been at work on a flora of the Rocky Mountain region, and in connection with his studies there have been many new species added and much critical work published. Therefore, when the Agricultural Experiment Station of Colorado was compelled to complete the determinations of its collections, it was natural to turn to Dr. RYDBERG, and the result is a station bulletin dealing with the flora of Colorado.[1] As was to be expected, the work grew in the preparation, so that it is nearly an exhaustive list of the plants at present known in Colorado. It is not a full descriptive manual in the ordinary sense, for under the species one finds only synonymy, range, and stations; but the analytical keys should enable one to determine the genus and species of all the forms ordinarily met. In this way 2,912 species of vascular plants are characterized, and this number is said to be surpassed by no state except California and perhaps Florida. It is quite characteristic of this great flora that one-fifth of it belongs to the Compositae, and that there are only twenty gymnosperms and forty pteridophytes. The nomenclature used by the author is well known, as are also his views on generic limitations. As he himself says in the introduction, he "belongs to that radical school which believes in small genera with closely related species, rather than in larger ones with a heterogeneous mass of different groups of plants having relatively little relationship to each other." The author also says that "the nomenclature used is in principle agreeing with the so-called American code adopted at a meeting in Philadelphia, and submitted to the International Botanical Congress at Vienna, with a few modifications resulting from a compromise with the European botanists." If this means that the Vienna Code is used only in so far as it meets the approval of the individual, then international congresses on nomenclature will be of little value until they achieve the impossible result of formulating a code that will satisfy all taxonomists. Very wisely the author publishes in this bulletin no new genera or species, or even new names or combinations. This publication will certainly serve a most useful purpose, and both author and station are to be commended for carrying it through.

The other flora is that of Washington by CHARLES V. PIPER[2]. The author says that his "principal aim is to present a summary of our present knowledge

[1] RYDBERG, P. A., Flora of Colorado. Agric. Exp. Sta. Colorado, Bull. 100. pp. xxii+414. 1906.

[2] PIPER, CHARLES V., Flora of the State of Washington. Contrib. U. S. Nat. Herb. 11:1–637. *pls. 1–22* and colored map. 1906.

of the vascular plants of Washington and to call attention to the more important problems, both taxonomic and ecological, which have become disclosed." The plan of the work is practically that of Dr. RYDBERG'S, namely a list of species with synonymy, range, etc., but with simple keys for general identification. The views as to the limitations of genera and species, however, are much more conservative, the author making the following interesting remarks: "It is at least doubtful if the very large number of new names thus occasioned does not more than counterbalance any advantage argued in favor of the practice. Certainly the carrying of the practice to such an extreme that genera are considered to be made up of species of similar habit, rather than to be based on structural character, seems inadvisable. Neither does it impress one as a valid argument that, because in some extremely natural families the genera must perforce be based on very slight differences, similar characters must be given equal consideration in all families." The pages given to an account of the botanical explorers of Washington, beginning with MENZIES, are most interesting, as is also the account of the physiographic features of the flora. The "annotated catalogue" comprises a very long list of vascular plants, and it is interesting to note that 185 of them are endemic, two of the genera included in the list (Rainiera and Hesperogenia) being monotypic. The number of gymnosperms is almost exactly that given above for Colorado, but the pteridophytes are more numerous, a list of 64 being given.—J. M. C.

AN INTRODUCTION to plant physiology by the LINSBAUERS[3] is very welcome and it is to be hoped that an English edition will be prepared. While too elaborate for our secondary schools at present, and yet too elementary for higher students, the work contains a great deal that may be efficiently adapted to any first course. The diction is semipopular. The first commendable feature one notes is the logical arrangement of the topics. The experiments (nearly 300) accompany the text, in fact are really a part of it. Following each chapter is a series of problems for independent investigation, so that each chapter first equips the student for independent work and then suggests that he do some as indicated. The difficult topics of semipermeability, osmosis, etc., are skilfully approached by preliminary experimentation with imbibition phenomena. Physical explanations involving such difficult subjects as solution-tension are very properly omitted. The treatment of some processes is far from modern. The combustion conception of respiration is developed. Photosynthesis is called "assimilation" and contrasted with respiration, which is also given the name of "dissimilation." This is a very unfortunate confusion of both terms and ideas. Of the seventy-eight cuts of the text proper, seven illustrate apparatus original in design.—RAYMOND H. POND.

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[3] LINSBAUER, LUDWIG, und LINSBAUER, Karl, Borschule der Pflanzenhpysiologie. Eine experimentale Einführung in das Leben der Pflanzen. 8vo. pp.—*figs. 78*. Carl Konegen, Vienna. 1906.

MINOR NOTICES.

Pflanzenfamilien.[4]—Part 226 contains the completion of the Neckeraceae and the beginning of the Lembophylaceae by V. F. BROTHERUS. The second part of the second supplement has also appeared, including the literature of 1899–1904 in reference to dicotyledons up to the beginning of Euphorbiaceae. —J. M. C.

NOTES FOR STUDENTS.

Archegoniatenstudien.—The tenth of GOEBEL'S series by this title is for size almost a book in itself. It is made up of over twenty papers on morphology and biology of mosses and liverworts, varying from a page or less, embodying a brief note on the water adaptation in the form and position of the leaves in Orthorhynchium, to a paper of forty-odd pages on Dawsonia and its allies, and a like one on marsupiferous Jungermanniales. In great part these papers were written several years ago, and some of the researches have been epitomized in GOEBEL'S *Organographie*, but they have not been published *in extenso* until now, on account of other work.

Together they form a most important contribution to our knowledge of the bryophytes—a contribution too full of details to report fully. At various places it has a tinge of the polemic, for the author has to clear away many errors, and he takes occasion to rebuke one and another for shortcomings. Much space is devoted to speculations, which are confessedly unsupported by investigation because material or time was lacking. Such speculations, if put briefly, may be suggestive as a guide to future investigations; but they appear to be indulged in as a basis for future claims to priority, if we may judge from some citations of earlier ones in these pages. Even a scientific man is rarely without a prejudice in favor of his own hypotheses. Thus the author guessed (*Organographie* 346) about the development of the multicellular "spores" of Dicnemon: "Am wahrscheinlichsten ist es dass sich aus den gekeimten Sporen ein Fadenprotonema bildet, etwa aus den Brutknospen von Tetraphis." Now he declares "dass die früher geäusserte Vermutung richtig war." But the "protonemal filaments" function "der Hauptsache nach als Rhizoiden;" rhizoids arise also from the surface; and the apical cell of the stem arises not as a branch of one of these "protonemal filaments" but almost immediately from a marginal cell of the "spore." It is difficult to see how the earlier guess can possibly be justified by these observations. Certainly the resemblance to the behavior of the gemmae of Tetraphis is rather remote. Other like instances might be cited; sometimes the guess was right, sometimes not; and that is likely to be the case with these new ones. But the observations are abundant, and the author's keen discrimination and clear presentation throw light upon many obscure points.

Dawsonia is held to be the primitive form of the Polytrichum line by reason of the limited differentiation of tissues in the axis of the gametophyte, and espe-

[4] ENGLER, A., und PRANTL, K., Die natürlichen Pflanzenfamilien. Lieferung 226. Leipzig: Wilhelm Engelmann. 1906.

cially on account of the structure of the peristome, whose development and anatomy he clears up. He also points out the relations of the peristome of the Buxbaumiaceae and Tetraphideae to that of the Polytrichaceae.

Dicnemonaceae (Dicnemon and Mesotus) are recognized as a natural group, characterized by the multicellular spores and the peculiar filamentous outgrowths on the leaves. which are considered as organs of water absorption. Both these characters and the structure of the sporophyte indicate adaptation for alternating dry and wet periods.

Leptostomum has a peristome corresponding to a degenerate mniaceous peristome. In Eriopus a fuller description is given of the leaves and of the rhizoids at the base of the sporophyte than in *Organographie* 377.

The symmetry of the leaves and their position on the axis is discussed at some length for the genera Pterygophyllum, Cyathophorum, Mittenia, Rhizogonium, and Orthorhynchium, and there are minor notes on sundry points.

The development of the leaves of several species of Gottschea is shown to be of the same type as in Fissidens; multicellular rhizoids are described and figured; the absence of a perigone is correlated with the boring of the embryo sporophyte deep into the stem; and *G. splachnophylla* shows a basal elaterophore like that of Pellia.

Paraphyllia were found in five genera, functioning in part for photosynthesis and in part for holding water.

The "Geocalyceae" are described at length, and for them the more appropriate designation marsupiferous Jungermanniales is suggested. Three types are discriminated: (*a*) Tylimanthus type (Tylimanthus, Marsupellopsis, Marsupidium), with pouch originally solid and hollowed out by the growing embryo; (*b*) Isotachis type, in which the archegonium after fertilization is surrounded by a ring-like wall arising from the stem tissues, which carries up the leaves; (*c*) the common type, with pouch arising after fertilization, hollow from the beginning (Balantiopsis, Acrobolbus, Lethocolea) There are intermediate forms between (*a*) and (*c*). In Acrobolbus there is even a "root-cap" on the pouch.

Another heterophyllous Radula, *R. uvifera*, is described, the so-called "slender male spikes" of hepaticologists being here, as in *R. pycnolejeunioides*, composed of small water sacs, frequently inhabited by animalcules; whereas the antheridia are protected by quite different leaves. Hymenophyllum, with its slender "stalk" and broader "leaf" shows no constancy in this differentiation, and since Pellia, Preissia, Fegatella, etc., show similar forms on being grown in the dark and then illuminated, the author is moved to conclude that "the stalk arises . . . . by autonomous etiolation"—a charming phrase which we owe to SACHS, but nevertheless a phrase which is merely a wordy cloak for ignorance.

The rudimentary "leaves" of *Blyttia xiphioides* are organs of protection for the apical region. The remarkable but inconstant water sacs of *Metzgeria saccata* arise, it is said, not by a lobing of the thallus, but by "an inrolling of the thallus margins from below and locally accelerated growth of isolated parts"—which latter sounds much like lobing in other words.

The liverworts furnish most instructive examples of parallel structures, of which the author cites many instances.—C. R. B.

Anaerobic respiration.—Inasmuch as PALLADIN and KOSTYTSCHEW, working independently, had agreed, contrary to the conclusions of several other observers, that anaerobic respiration was not identical with alcoholic fermentation, it seemed good to them to reinvestigate the question. They now find[5] that while not identical in all plants and under all conditions, there are striking coincidences. For example, in living lupine seeds and seedlings they consider the anaerobic respiration identical with alcoholic fermentation; but in frozen lupine seedlings and stem tips of *Vicia Faba* the former has nothing to do with the latter. In pea seeds and wheat embryos, living and frozen, there occurs a considerable formation of alcohol, and the anaerobic respiration is "in great part" alcoholic fermentation. They confirm the results of GODLEWSKI, STOCKLASA, and others regarding the presence of "zymase," but think it yet remains to be shown that it is identical with yeast zymase. Under certain conditions aceton and its allies are formed, both in aerobic and anaerobic respiration of living and frozen plants.

It becomes more and more evident that the course of the respiratory decomposition of the protoplasm may be varied.—C. R. B.

Thermal death-point.—MEYER[6] has determined a formula by which may be calculated the time necessary to kill bacteria at any given temperature, when observation has determined the time necessary at any two convenient temperatures, such as 80° and 100°. This rests upon the observation that the death periods form a geometrical progression, decreasing with the increasing temperatures. Thus the formula is $q = \sqrt[n-1]{\frac{a}{t}}$, in which a is the first member of the progression, t any other known member, q the progression, n the number of terms. Thus, BLAU had determined the death period of *Bacillus subtilis* at 100° as 180 minutes, and at 80° as 4500 minutes. Whence $q = \sqrt[2]{\frac{180}{4500}} = 0.2$. The calculated series then would be: 80°, 4500 minutes; 90°, 900 minutes; 100°, 180 minutes; 110°, 36 minutes; 120°, 7.2 minutes; 130°, 1.4 minutes; 140°, 0.28 minutes or 17 seconds; 150°, 3.4 seconds. The figures observed by MEYER agree well with these calculations. In practice this has an important application in enabling one to calculate the supramaximal temperature, as ENGELMANN called it, i. e., the time necessary to kill any form instantly—say in one second.—C. R. B.

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[5] PALLADIN, W., and KOSTYTSCHEW, S., Anaerobe Atmung, Alkoholgärung und Acetonbildung bei den Samenpflanzen. Ber. Deutsch. Bot. Gessels. **24**:273–285. 1906.

[6] MEYER, ARTHUR, Notiz über eine die supramaximalen Tötungszeiten betreffende Gesetzmässigkeit. Ber. Deutsch. Bot. Gesells. **24**:340–52. 1906.

Geotropic stimulation and position.—CZAPEK[7] has replied to FITTING with a paper which is largely a comparative study of the methods and results of the workers in this problem. The author concedes to FITTING that in many cases stronger stimulation occurs at 90° than at 135°. On the other hand, FITTING's conclusion that 45° above the horizontal and 45° below are equivalent positions is rejected. The reaction time is found to be practically the same at deviations between 20° and 160°, but is noticeably longer either above or below those limits. The method of anti-ferment reaction shows that the stimulation is clearly less at 45° below than at 45° above. In the inverse position there is no anti-ferment reaction. Just how much significance is to be attributed to the results of this method the reviewer cannot say. CZAPEK believes that in spite of all the investigation of this problem a satisfactory solution is still in the future.—RAYMOND H. POND.

Chemistry of germination.—ZALESKI has studied certain changes that occur in the proteids of germinating seeds and contributes these points. The phosphorus-containing proteids and phosphatids (chiefly lecithin) are very quickly and almost totally decomposed by an enzyme, with the formation of "inorganic" phosphates, only 2 per cent. remaining unattacked. These bodies are apparently nucleo-albumins (phytovitellins). What the enzyme is, whether trypsin or a special one, remains to be investigated.[8] The formation of asparagin, like the proteid decomposition, is an enzymic process, proteolysis yielding material which forms asparagin in an unknown way and independent of temperature changes, at least in the later stages of germination. The nature of this process is to be further studied by the author.[9]—C. R. B.

Absorption of solutes by soils.—Bulletin 32 of the Bureau of Soils[10] is consistent with the high standard established by the previous publications of the Bureau. SCHREINER and FAILYER find as a general law in the case of phosphates that the amount of solute a given soil will withdraw from solution percolating through it is proportional to the quantity which the soil is still capable of absorbing.—RAYMOND H. POND.

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[7] CZAPEK, FRIEDRICH, Die Wirkung verschiedener Neigungslagen auf den Geotropismus parallelotroper Organe. Jahrb. Wiss. Bot. **43**:145–175. 1906.

[8] ZALESKI, W., Über die Rolle der Enzyme bei der Umwandlung organischer Phosporverbindungen in keimenden Samen. Ber. Deutsch. Bot. Gesells. **24**:285–291. 1906.

[9] ———, Zur Frage über den Einfluss der Temperatur auf die Eiweisszersetzung und Asparaginbildung der Samen während der Keimung. Ber. Deutsch. Bot. Gesells. **24**:292–5. 1906.

These two titles are excellent examples of over-minuteness—a fault to be avoided for the sake of those who have to cite the papers in future years.

[10] SCHREINER, OSWALD, and FAILYER, GEORGE H., The absorption of phosphates and potassium by soils. Bureau of Soils, U. S. Department of Agriculture, Bull. 32. 1906.

Scion and stock.—By grafting *Nicotiana Tabacum* on *N. affinis* (which contains little or no nicotin), and *N. affinis* on *N. Tabacum*, GRAFE and LINSBAUER have succeeded in showing,[11] in a more convincing way than before, the effect of the scion on the stock in respect to products of metabolism. Nicotin was found abundantly in *N. affinis*, whether it was functioning as stock or scion. Indeed, it attained almost the maximum amount found in *N. Tabacum* and scarcely fell below the limits of variation in that species. When *N. Tabacum* was the stock, and the scion, *N. affinis* was cut away completely, the new shoots produced contained even less nicotin than the *N. affinis* leaves had; so that the authors believe the scion had even increased the capacity of the *N. Tabacum* stock to form this alkaloid. Further researches are in progress.—C. R. B.

Tobacco.—In a long and somewhat controversial paper, excellently illustrated by halftones of various races of tobacco, ANASTASIA[12] concludes that there are four varieties within which may be grouped all the races of commerce. Three of these, vv. *havanensis*, *brasiliensis*, and *virginica*, are the offspring of *Nicotiana Tabacum*, and one owes its origin to hybridization between *N. Tabacum* and an unknown species of Nicotiana. Dr. ANASTASIA is desirous of securing seeds of certain races cultivated in the U. S. We bespeak the cooperation of those living in tobacco-raising sections. He may be addressed at the Experiment Station, Scafati, Salerno, Italy.—C. R. B.

Phototropism.—Further proof that the epidermal cells of phototropic leaves act as lenses, thus enabling them to function as receptive organs for adjustments to light, is adduced by HABERLANDT[13]. On covering young leaves of *Begonia semperflorens* with a layer of water, held in place by thin mica, he found no response to oblique light, though control leaves had attained the usual transverse posture, and the water-covered leaves gained it, though not perfectly, after removal of the layer of water.—C. R. B.

Solution cultures.—BREAZEALE[14] finds that transpiration and size of wheat seedlings are increased by the presence of sodium in nutrient solutions containing all other necessary elements. Further, the previous presence of sodium in a nutrient solution decreases the amount of potassium entering the plant during a subsequent period. The paper shows very little consideration for the reader. RAYMOND H. POND.

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[11] GRAFE, V., and LINSBAUER, K., Über die wechselseitige Beeinflussung von *Nicotiana Tabacum* und *N. affinis* bei der Pfropfung. Ber. Deutsch. Bot. Gesells. **24**:368–71. 1906.

[12] ANASTASIA, G. EMILIO, Le varietà typiche della *Nicotiana Tabacum* L. R. Istituto Sperimentale Tabacchi in Scafati. Ministero delle Finanze. Imp. 8vo. pp. 122. *figs.* and *plates 31*. 1906.

[13] HABERLANDT, G., Ein experimentaler Beweis für die Bedeutung der papillosen Laubblattepidermis als Lichtsinnesorgan. Ber. Deutsch, Bot. Gesells. **24**:361–6. 1906.

[14] BREAZEALE, J. F., The relation of sodium to potassium in soil and solution cultures. Journ. Amer. Chem. Soc. **37**:1013–1025. 1906.

NEWS.

DURING 1905 Kew Herbarium received in gifts over 16,000 sheets from about one hundred persons and institutions, and purchased nearly 7,000 sheets.

RAYMOND H. POND, Northwestern University, has been awarded a research scholarship at the New York Botanical Garden for six months, beginning on October 1.

THE BOTANICAL DEPARTMENT of the University of Illinois has purchased the herbarium of GEORGE D. MCDONALD, of Peoria, Ill. It contains about 12,000 specimens.—*Science*.

VERNON H. BLACKMAN, for ten years in the Department of Botany of the British Museum, has resigned this position to become Lecturer in Botany at the Birkbeck Institute. He also holds a lectureship at the East London College.

IN *Botanisches Centralblatt* (**102**: 367. 1906) there is published a short biographical sketch of the late Professor H. MARSHALL WARD, prepared by Professor S. H. VINES; and another notice appears in the *Kew Bulletin* (1906: 281), by L. A. BOODLE.

AN APPRECIATIVE NOTICE of the life and work of the late C. B. CLARKE appears in *Bulletin de l'Herbier* for September 1906, prepared by CASIMIR DE-CANDOLLE. Another biographical sketch of CLARKE, unsigned and including bibliography, is published in *Kew Bulletin* (**1906**: 271–281).

IN *Journal of Botany* for October 1906 there appears a biographical sketch of WILLIAM MITTEN, the bryologist, prepared by W. B. HEMSLEY, and accompanied by an excellent portrait. He died July 27, 1906, in his eighty-seventh year. The same number also contains a portrait of ROBERT BROWN. Another sketch of MITTEN by Helmsley is published in *Kew Bulletin* (**1906**: 283).

A GENERAL ACCOUNT of the work of Section K at the York meeting of the British Association is published in *Nature* of October 4. There were three appointed discussions upon the following topics: Some aspects of the present position of paleozoic botany, opened by D. H. SCOTT; The nature of fertilization, opened by V. H. BLACKMAN; The phylogenetic value of the vascular structure of seedlings, papers being read by a number of botanists whose names are identified with this phase of work.

WITH THE first part of volume 96, issued late in March, the publication of *Flora* passed into the hands of the well-known house of GUSTAV FISCHER. Hereafter the volumes will be enlarged to at least 560 pages, without increase in price, and the designation of *Erganzungsbände* will be abandoned. Fortunately they were always numbered consecutively with the others, and so the superfluous name made little bibliographic confusion. No reviews of literature are to appear in future. Articles are to be restricted in length as a rule to 48 pages, and for this the editor clears his desk by getting in the tenth of his *Archegoniatenstudien* as the leader of the new volume, a paper of over 200 pages!